Supplementary Information for

Ancient genomes from present-day France unveil 7,000 years of its demographic history.

Samantha Brunel, E. Andrew Bennett, Laurent Cardin, Damien Garraud, Hélène Barrand Emam, Alexandre Beylier, Bruno Boulestin, Fanny Chenal, Elsa Cieselski, Fabien Convertini, Bernard Dedet, Sophie Desenne, Jerôme Dubouloz, Henri Duday, Véronique Fabre, Eric Gailledrat, Muriel Gandelin, Yves Gleize, Sébastien Goepfert, Jean Guilaine, Lamys Hachem, Michael Ilett, François Lambach, Florent Maziere, Bertrand Perrin, Susanne Plouin, Estelle Pinard, Ivan Praud, Isabelle Richard, Vincent Riquier, Réjane Roure, Benoit Sendra, Corinne Thevenet, Sandrine Thiol, Elisabeth Vauquelin, Luc Vergnaud, Thierry Grange, Eva-Maria Geigl, Melanie Pruvost

Email: thierry.grange@ijm.fr, eva-maria.geigl@ijm.fr, melanie.pruvost@u-bordeaux.fr,

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SI.1 **Archaeological context**

In order to thoroughly cover the different periods encompassed in the 7,000-year transect of this project, a total of 243 samples were processed (Dataset1). The sampling favored temporal bones when available, resulting in the following distribution of sampled materials:

- 232 temporal bones,
- 3 long bones,
- 8 teeth.

Most of the sampled individuals spanned the Neolithic to the Iron Age (~5500 to 25 BCE) and were collected from three regions of the present-day France: 117 from the Grand-Est (East), 99 from Occitanie (South) and 22 from the Hauts-de-France (North). The remaining five samples were associated with the Mesolithic period and sampled from Nouvelle-Aquitaine (South-West), a region outside the geographic regions initially encompassed in the project.

In this section we will briefly describe the archaeological context of each sample sequenced in this study. We will specify dates in one of two formats. For example if there is no direct radiocarbon date on the individual analyzed with aDNA, we give a date based on the archaeological context or on the genetic results in the following format: "7700–7100 BCE". Alternatively, if there is a direct radiocarbon date on the analyzed bone, we show the resulting calibrated date as 7458–7364 cal BCE.

40 new carbon-14 dates have been generated for this study and are listed in the Dataset S2. Most of them have been dated through the ARTEMIS program (MMC) in the C-14 laboratory of Lyon.

The locations of the archaeological sites included in the study are shown on the map Figure S1-1 and their chronologies are presented on three different timelines for the three regions "Alsace", "South-East" and "Champagne-North" (Figure S1-1).

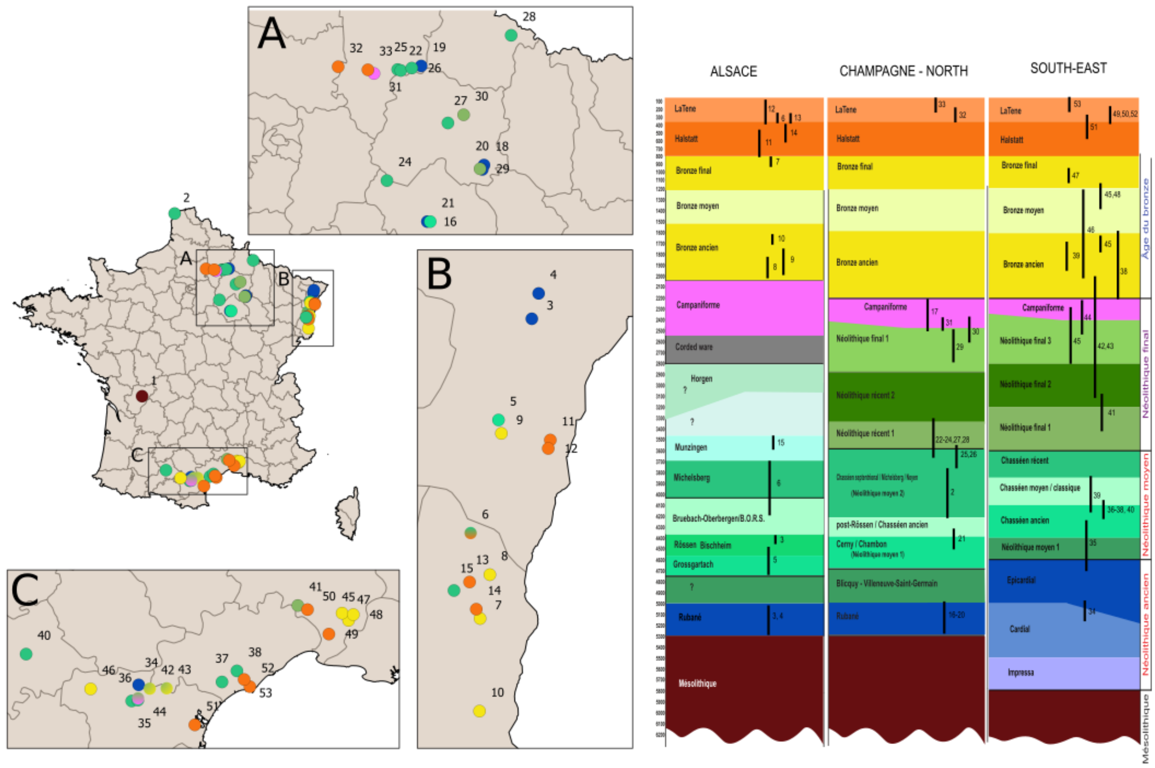


Figure S1-1: Locations of the archaeological sites included in the study and their chronologies presented on three different timelines for the three regions "Alsace", "South-East" and "Champagne-North". 1- La Grotte des Perrats (Agris, Charente) 2- Escalles (Mont d'Hubert, Pas-de-Calais) 3- Les terrasses de la Zorn (Schwindratzheim, Bas-Rhin) 4- Ungeheuer Hoelzle (Morschwiller-Le-Bas, Haut-Rhin) 5-Rosheim Mittelfeld (Rosheim, Bas-Rhin) 6- Saulager (Bergheim, Haut-Rhin) 7- Innere Allmende (Niederhergheim, Haut-Rhin) 8- Muelhaecker (Bischwihr, Haut-Rhin) 9- Parc d'activités économiques intercommunal (Obernai, Bas-Rhin) 10- ZAC du Petit Prince (Rixheim, Haut-Rhin) 11- Buerckelmatt (Nordhouse, Bas-Rhin) 12- Untergasse (Erstein, Bas-Rhin) 13- Jardin des Aubépines (Colmar, Haut-Rhin) 14- Jebsenboden (Sainte-Croix-en Plaine, Haut-Rhin) 15- Ricoh (Wettolsheim, Haut-Rhin) 16- Saint-Léger-près-Troyes17- Les Pointes et les Grèvottes "ZAC St Martin 1" (Breviandes, Aube) 18- Les Noues, Journée Carrée (Orcontes, Marne) 19- Derrière le Village (Menneville, Aisne) 20- Le Champ Buchotte (Larzicourt, Marne) 21- (Buchères, Aube) 22- La Plaine (Beaurieux, Aisne) 23- Le Haut de Launois, Ferme de l'île (Pont-sur-Seine, Aube) 24- Pré Chevalier (La Villeneuve au chatelot, Aube) 25- Les Fontinettes, Champ Tortu (Cuiry-lès-Chaudardes, Aisne) 26- la Croix-Maigret (Berry-au-Bac, Aisne) 27- Recy (Châlons-en-Champagne, Aube) 28- Les Hautes Chanvières (Mairy, Ardennes) 29- Le Prieuré (Isle-sur-Marne, Marne) 30- Chemin dit des Royats (La Cheppe, Aube) 31- La Bouche a Vesle 32- Attichy 33- Bucy le long 34- La grotte Gazel (Sallèles-Cabardès, Aude) 35- Le Champ du Poste (Carcassonne, Aude) 36- Les Plots (Berriac, Aude) 37- Le Crès (Béziers, Hérault) 38- Le Pirou (Valros, Hérault) 39-Villeneuve-Tolosane (Haute Garonne) 40- Cugnaux (Haute Garonne) 41- Aven de la Boucle (Corconne, Gard) 42- Le dolmen de Saint-Eugène (Laure-Minervois, Aude) 43- Le dolmen des Fades (Pépieux, Aude) 44- Le dolmen des Peirières (Villedubert, Aude) 45- Mitra 2 and 3 (Saint-Gilles-du-Gard, Gard) 46- Quinquiris (Castelnaudary, Aude) 47- Mas de Vignole IV (Nimes, Gard) 48- Manduel 49- Le Cailar 50-

Oppidum du Plan de la Tour (Gailhan, Gard) 51- Oppidum de Pech Maho (Sigean, Aude) 52- La necropole du Peyrou 2 (Agde, Herault) 53- La Monedière (Bessan, Herault)

1- La Grotte des Perrats[1,2] (Agris, Charente) – B Boulestin

Les Perrats cave, located in Agris, département of Charente, was discovered in 1981. Excavations were first carried out from the date of the discovery until 1994, then later from 2002 to 2008. The site has yielded one of the longest post-palaeolithic stratigraphic sequences in Western France, the earliest known occupation dating back to the end of the First Mesolithic, the last to the Late Middle Ages.

Highly fragmented and scattered human bones, belonging to at least eight individuals –five adults and three children– were discovered mixed with faunal remains at the cave entrance in the First Mesolithic levels. These levels testify to one or several, likely brief occupations, radiocarbon dated to the last third of the 8th millennium BCE. The lithic artefacts found along with the bones belong to the archaeological culture called central-western Middle Mesolithic and/or to the Sauveterrian from the Quercy region.

Most of the human remains display anthropogenic modifications, pertaining either to corpse cutting or to bone breaking operations. The modifications of the various skeletal parts is largely similar to the butchery practices observed on the faunal remains, indicating a functional exploitation aimed at food extraction. The human remains are consequently those of victims of cannibalism.

2- Escalles (Mont d'Hubert, Pas-de-Calais) – I. Praud

Recent excavations at Escalles in the Pas-de-Calais have uncovered a causewayed enclosure dating to around 4000 cal BC. The enclosure is located on the Mont d'Hubert, less than 1 km inland from Cap Blanc Nez. At 150 m, this hill dominates the surrounding landscape and offers stunning views of the Dover Strait and the Kent coastline. Excavations revealed a 120 m stretch of causewayed ditch, barring the widest part of the chalk spur and enclosing a surface area estimated at 4.5 ha, bordered on the west by an abrupt slope. There are four ditch segments, on average 3.50 m wide and 1 m deep. The finds from the enclosure ditch reflect both abundant use of locally available resources and intensive social activity. The ditch fill contained over 1500 kg of flint, 500 kg of sandstone, 174 kg of pottery, 160 kg of faunal remains (mostly from domestic cattle, caprines and pig), as well as around 100 bone and antler tools and 3865 litres of shells of the marine species *Mytilus edulis* and *Patella*. Carbonised plant remains include cereal grains and hazelnuts. Roughly 2000 fragments of human bone were found, with numerous small fragments. These show signs of breakage of fresh bone, including skulls, together with cut-marks from defleshing or dismemberment. Some fragments have signs of exposure to fire. Altogether the human bones correspond to nine adults and eight subadults.

3- Les terrasses de la Zorn (Schwindratzheim, Bas-Rhin) – B. Perrin and A. Denaire

The city of Schwindratzheim is located about 20 km north-west of Strasbourg. The site shows evidence of several Neolithic and Protohistoric occupations.

The best represented period is the ancient Neolithic. Three houses could be identified, and possibly a fourth. Two burials have been discovered near these buildings. The dead have their limbs flexed and their heads facing south-east. The other structures refer for the most part to the category of multi-lobed pits. Of these, one is distinguished by the presence of human remains belonging to at least six individuals (one adult and five sub-adults).

The occupation of the Middle Neolithic includes remains attributed to the Bischheim group. About 80 m northwest of the ditches, two Bischheim burials were discovered. A grave contains the remains of a woman over 50 years old. Less than a meter to the northwest, a child or adolescent was buried in a large oval pit.

4- Ungeheuer Hoelzle[3] (Morschwiller-Le-Bas, Haut-Rhin) – L. Vergnaud

The first traces of a postglacial human occupation on the Morschwiller-le-Bas "Ungeheuer Hoelzle" (Haut-Rhin) site, excavated during the summer of 2013, date back to the ancient LBK and consist of a set of several burials.

The site is composed of five graves, close to each other. Three of them are aligned along a north-east/south-west axis. Two other burials are located directly southeast of the three aligned graves.

The bones of the skeletons are rather well preserved. The individuals are arranged with their heads to the east and in the folded position on the left side or on the right side with the lower limbs flexed. Some objects accompanied the deceased and two of the tombs contained many ornaments. The set of funerary practices observed in Morschwiller-le-Bas is broadly consistent with the funerary tradition I characteristic of the Haute-Alsace Rubané.

5- Rosheim Mittelfeld[4] (Rosheim, Bas-Rhin) – F. Chenal

The city of Rosheim is located about 25 km west of Strasbourg in the Bas-Rhin. It has yielded numerous archaeological remains, from the Neolithic to the Gallo-Roman period. A vast necropolis, used continuously from 4800 to 4700 BCEE, was discovered in 1996 and was the subject of several campaigns of rescue excavations under the direction of Christian Jeunesse (1998) and Eric Boës (1999 and 2000). With 112 individuals unearthed for the Grossgartach and Roessen culture, the Rosheim necropolis is currently one of the most important sites in France for this period.

The funerary practices identified in Rosheim are comparable to those traditionally observed for the Grossgartach culture. The deceased are buried on the back, the lower limbs extended and accompanied by grave goods within individual primary burials (mainly ceramics, milling elements, objects in bone industry, ornaments, lithic tools and dyes).

The burial complex is composed of correspond offamily groups of adults and subadults, men and women.

6- Saulager[5] (Bergheim , Haut-Rhin) – B. Perrin

The Bergheim site (Haut-Rhin - France) was excavated in 2012 by Antea-Archéologie. This archaeological operation, covering an area of approximately two hectares, revealed the existence of several Neolithic and protohistoric occupations.

60 silos could be attributed to the Middle Neolithic, 14 of which yielded human remains. These remains correspond to complete individuals (individual or multiple deposits), to anatomical parts or to isolated bones, with different categories found within the same structure. One of the site's structures, Silo 157, stands out for its exceptional character.

This pit contained mainly human remains and was dated around 4000 BCE. At its base were seven portions of left upper limbs amputated at the arm, still partially in connection. Directly above, eight bodies were stacked, in variable positions that could be described as "disordered", thus appearing to have been deposited without any particular organization. They belong to two men, two women, and four children. The lowest individual, a man aged 30 to 59, also had his left upper limb amputated. In addition to the traces of cutting blows related to amputations, the upper limbs have cutting marks and the amputated individual has several traces of violent blows, especially on the head, which most likely correspond to his killing.

7- Innere Allmende[6] (Niederhergheim , Haut-Rhin) – S. Goepfert

The municipality of Niederhergheim is located about 10 km south of Colmar, on the western margin of the ello-rhenish plain, near the Piedmont Vosges. The excavation of the "Innere Allmende" site in 2012 uncovered several signs of human occupation between the early Bronze Age and the Roman period. One of them corresponds to a necropolis with circular enclosures of the end of the Bronze Age which has the peculiarity of being associated with a Langgraben monument (elongated pen). Despite this funerary context, very few tombs were discovered due to massive erosion.

8- Muelhaecker[7] (Bischwihr, Haut-Rhin) – L. Vergnaud

During the excavation of the site of Bischwihr "Muelhaecker" (Haut-Rhin) during winter 2011, several domestic occupations dating from different periods, mainly protohistoric, were discovered. However, the oldest remains discovered belong to a small burial complex dated from the early Bronze Age. Nine burials, located approximately in the center of the right-of-way, were arranged on a strip about 15 m long and almost 5 m wide, roughly oriented along an east-south-east/west-north-west axis. They contained the remains of ten individuals (a newborn, four children and five adults), without any funeral endowment. The bodies of the deceased were placed in a bent position (or at least with their legs folded) on the right side with their head to the east, or to the left with their head to the west. The two confirmed women are among the individuals buried on the right side. The poor state of preservation of the bone material allowed sex determination only in the case of two women. Various clues lead us to consider, for at least three burials, the existence of a wooden architecture.

A series of six radiocarbon dates were made, but only one gave a result: Poz-47224: 3570 ± 35 BP, ie 1973-1882 BCEE at 1 σ (Sep 183).

9- Parc d'activités économiques intercommunal[8] (Obernai, Bas-Rhin) – F. Chenal (RO : C. Féliu)

The town of Obernai extends in Center Alsace, at the foot of the Vosges, about 20 km southwest of Strasbourg (Bas-Rhin). The excavation, conducted by Inrap under the direction of C. Feliu in 2013 on a surface of 7.5 Ha, has yielded many archaeological remains dating from the Neolithic to modern and contemporary periods. Among them a small burial complex dating from the early evolved Bronze Age as well as two burials of La Tène have been discovered.

The small group from early Bronze Age consists of six burials, including one containing seven individuals. The deceased are buried on the side with the limbs flexed, as is traditionally observed in the region for this period. The corpus is composed of two adult individuals, a man and a woman and five subadult individuals. Grave goods are scarce. However, adults are buried with bronze items that might suggest an eastern origin for the female individual and a Danubian origin (middle Danube) for the male individual. Their presence in Alsatian burials highlights the links between the southern Upper Rhine Plain and the Danubian regions during this chronological horizon.

The two individual primary burials dated to La Tene contain the remains of two adults, a man and a woman. This type of structure is rare in Alsace and the Upper Rhine for this period, for which cremation dominates almost exclusively. In addition, they have been found within a living area, which is also rare for the region.

10- ZAC du Petit Prince[7] (Rixheim, Haut-Rhin) – L. Vergnaud et Ph. Lefranc

The six tombs of Rixheim are organized linearly along a north-west/south-east axis, developing over 13 m in length.

All tombs contain primary deposits of five juveniles and one adult male. The sepulchral pits have sub-circular to oval shapes, vertical walls and flat floors. Their dimensions are adjusted to the size of the subject buried. No trace of funerary architecture has been detected. All skeletons are in the lateral position, lying on the right or left side, lower limbs flexed and upper limbs drawn towards the face. The four individuals deposited on the right side, all juveniles, are oriented on a west-east axis, with their heads to the west. The adult and the last child were placed on the left side, on an east-west axis, with their heads to the east.

The tombs do not contain any grave goods, AMS dating was performed on two of the skeletons. The results obtained (GrA-33880: 3415 + 40 BP is 1880-1610 Av at 2 σ, GrA-33881: 3375 + 40 BP is 1760-1530 at 2 σ) anchor the use of the necropolis to an evolved stage of the early Bronze Age.

11- Buerckelmatt[9,10] (Nordhouse, Bas-Rhin) – S. Plouin, F. Lambach

Located about 20 km south of Strasbourg, in the alluvial plain of l'Ill, the necropolis of Nordhouse is formed of six Tumuli. They are mostly dated from the late Bronze Age IIIb, with a cremation deposited in the center of a ditched circular enclosure. Accompanied by a rich ceramic items, cremated human remains are placed within a wooden architecture and pottery characteristic of the same phase was found in the eastern sector of each ditch.

During the first and early Iron Ages, numerous weed burials were later deposited in the tumulus, with each burial monument having between 14 and 27 graves. A total of 101 burials were found. The necropolis of Nordhouse was occupied between the 7th and 4th century BCE (between Hallstatt C2 and La Tène B1). An exceptional female tomb stands out by the abundance and richness of its grave goods, such as glass, amber, coral, a large shell from the Red Sea and many gold ornaments.

12- Saint-Léger-près-Troyes – C. Paresys

As part of a construction project, 13,000 m2 were excavated by Inrap, in 2011, in the commune of Saint-Léger-près-Troyes, 10 km south of Troyes, in the heart of the valley of the Seine (Aube). This excavation revealed 650 fossil vestiges related to ten occupation stages from the late Mesolithic to the modern era. Among these remains, burials occur only twice: the first in the early Neolithic with individual burials and the second during the transition late Bronze Age to early Iron age with cremations.

13- Les Pointes et les Grèvottes "ZAC St Martin 1" (Breviandes, Aube) – C. Laurelut

The "Rubané" site of Bréviandes "ZAC St Martin 1" is located on an old terrace of the Seine, overlooking the current alluvial plain (Aube). The occupation appears quite substantial (between six to twelve houses). This pioneering site appears to have been established at the end of the Middle "Rubané" (around 5200 BCE), with the bulk of the occupation concerning the beginnings of the recent "Rubané". This is the oldest Neolithic occupation of the Seine Valley. In addition to the houses and associated pits, the site includes five ancient Neolithic burials, including a multiple burial (badly degraded by plowing). The radiocarbon dates of the burials are rather low (towards 5000 BCE), suggesting their possible interment after the abandonment of the village. One grave located in the center of the site is radiocarbon dated to the "Cerny" culture (towards 47000 / 4500 BC).

Three small burial zones are scattered on the northern, southern and western limits of the "Rubané" occupation, each consisting of a small collective burial, all of which are heterogeneous in their structure as in the funerary practices. Two burial "annexes" are associated with the burial 445. On the basis of radiocarbon dates, these burials are related to one at the extreme end of the recent Neolithic, around 3100/2900 BCE, the others being clearly in the late Neolithic, around 2800/2500 BC.

14- Derrière le Village[11] (Menneville, Aisne) - C. Thevenet, M. Ilett

Menneville "Derrière le Village", 20 km north of Reims, is the easternmost final Linear Pottery (LBK – Early Neolithic) settlement in the river Aisne valley. Unlike the other LBK sites here, the settlement is surrounded by a large interrupted ditch, enclosing a surface area of 6.4 hectares.

Rescue excavations on the eastern third of the site (1989-1990) uncovered eight LBK floorplans and sixteen burials, located either alongside the houses or clustered just south of the enclosure ditch. Further human remains were found in the ditch, varying from isolated bones to primary burials of sixteen individuals (Thevenet 2016; 2017). The latter include two complex multiple burials of young children. These ditch burials are associated with placed deposits of caprine and cattle bones, including bucrania. Radiocarbon dates on human bone from the settlement and ditch burials include Lyon-3566 [SacA-5460] 6110 ± 30 BP, 5203-4947 cal BCE (st. 93); Lyon-3567 [SacA-5461] 6070 ± 30 BP, 5049-4857 cal BCE (st. 188); Lyon-3568 [SacA-5462] 6090 ± 30 BP, 5193-4938 cal BCE (st. 248); Lyon-3570 [SacA-5464] 6055 ± 30 BP, 5033-4850 cal BCE (st. 254).

Since 2013, research excavations have been carried out on the central and western parts of the LBK enclosure, providing a range of new evidence for funerary use of the ditch.

15- Buchères (Buchères, Aube) – C. Paresys

Several Neolithic occupations have been discovered on the Buchères (Aube) excavation in 2012.

For the early Neolithic, an isolated settlement of “Rubané” and a housing area of “Blicquy / Villeneuve-Saint-Germain (BVSG)” were discovered accompanied by a small group of individual burials.

The Middle Neolithic I/Cerny necropolis of about fifteen individual burials is link with a small funerary monument. Elements of adornment (shell beads, limestone, and animal teeth) as well as flake laminar were discovered associated with the deceased. No element seems to be related to Middle Neolithic II, corroborating the absence of data already observed in previous campaigns, which gave the impression of a prolonged abandonment of the sector until the recent Neolithic period.

16- La Plaine[12] (Beaurieux, Aisne) - C. Colas

Beaurieux "La Plaine" is located in the Aisne valley, 35 km east of Soissons. It is a Middle Neolithic funerary site comprising two or three Passy-type monuments, one of which contains burial 31 (radiocarbon date GrA-35371: 5775 ± 45 BP 4722-4515 calBC), as well as a massive structure over 80 m long, unprecedented in terms of its trapezoidal groundplan, dimensions and association with Passy-type monuments.

The site also produced five isolated graves attributed to Michelsberg, as well as a hairpin-shaped monument enclosing two graves dated to the Michelsberg both by radiocarbon dates and abundant grave goods (burial 9 - GrA-31115: 5370 ± 40 BP 4332-4046 calBC; burial 10 - GrA-3117: 5275 ± 40 BP 4224-3980 cal BCE and GrA-3119: 5375 ± 40 BP 4333-4047 calBC).

The site thus forms a new kind of funerary complex for the region, showing use of an area for burial over a long period of time and suggesting transmission from Cerny to Michelsberg of the practice of placing graves within monuments, the shapes of which are quite clearly related.

17- Le Haut de Launois, Ferme de l'île (Pont-sur-Seine, Aube) – I. Richard, S. Desbrosse-Degobertière

The site of Pont-sur-Seine "Ferme de l'île" is located in the Aube, about 10 km north-east of Nogent-sur-Seine, in the Seine Valley. The occupation of the site begins in the Neolithic, and is illustrated by a ditched enclosure, ditches, structures on poles and pits. It continues into Protohistory then into Gallo-Roman, medieval, and modern times.

About 50 Neolithic burials have been discovered. The clustered graveyards seems to have developed around older burials, presumably associated with the living enclosure. The palisaded enclosure belongs to the "Groupe de Noyen", a cultural entity linked to the "Michelsberg" culture. The graves show great variation of their form, size, depth and complexity. Both sexes are identified and children are present in quite a large number (20 children). The burials contain grave goods of ceramics, bone tools, lithic pieces, ornaments, fauna or stones, but some are devoid of funerary deposits.

18- Les Fontinettes, Champ Tortu[13] (Cuiry-lès-Chaudardes, Aisne) - L. Hachem, C. Colas

The multi-period site at Cuiry-lès-Chaudardes "Les Fontinettes", located in the Aisne valley 35 km east of Soissons, includes a large Michelsberg settlement (Middle Neolithic 2). In contrast to contemporary monumental ditched enclosures, this occupation consists of one hundred or so pits of varying shape and depth, widely scattered over a surface area of around 5 hectares and partly enclosed by a palissade. The pottery typology suggests that the occupation covers two stages of the Michelsberg culture. Two graves were also found. These have been recently radiocarbon dated.

The date from the adult burial 488 (GrN-28267: 4980 ± 50 BP, 3940-3650 calBC), places this in the later Michelsberg. The other grave (st. 315) is a double child burial and produced an even later date, corresponding to a possible "Middle Neolithic 3" (Ly2727 [Po] 4800 ± 45 BP, 3660-3510 calBC).

Cuiry-lès-Chaudardes "Champ Tortu" is located in the Aisne valley 36 km east of Soissons (Colas *et al*. 2015). This is a Middle Neolithic occupation consisting of four to seven structures of polygonal shape, one pit, a partially preserved archaeological layer and two burials (st. 99, st. 112). Three Late Neolithic cremations were found in another part of the site.

Burial 99 produced a radiocarbon date comparable to other dates for the Aisne valley Michelsberg (GrA-47885: 5040 ± 40 BP, 3953-3714 cal-BCE). The date from burial 112 (GrA-47880: 4785 ± 40 BP, 3638-3384 cal-BCE) is later and could either be latest Michelsberg or represent an intermediary stage with the Late Neolithic. This is consistent with the dating evidence from the one pit on the site. It contained some typical Michelsberg vessels, together with some less diagnostic pottery and two conical weights usually associated with later cultures. The radiocarbon date from burial 112 also falls within the range of dates obtained for the polygonal structures, suggesting they may be associated and raising the question of the nature of domestic structures for this particular chronological stage.

19- la Croix-Maigret (Berry-au-Bac, Aisne) - J. Dubouloz

Burial 137 at Berry-au-Bac "la Croix-Maigret", from a grave tangent to the palissade of the late Rössen enclosure (c. 4400-4300 BCE), was originally thought to date to just after this late Middle Neolithic 1 occupation, but a recent, radiocarbon date (Lyon3560 [SacA-5454] 4695 ± 30 BP, 3627-3371 cal BCE) brought the dating forward to the second half of the 4th millennium BCE, a substantially after the enclosure. The general context is thus an isolated grave, with no traces of settlement nearby. Throughout the Paris basin, there are increasing numbers of graves dated to c. 3600-3400 BCE and they contribute to the identification of a "Middle Neolithic 3", precursory to the Late Neolithic and poorly documented in terms of settlements.

20- Recy (Châlons-en-Champagne, Aube) – I. Richard

The town of Recy is located in the Marne department, northwest of the city of Châlons-en-Champagne. The excavation took place in 2013 and 2014 under the responsibility of Nathalie Achard-Corompt, on an area of 7 ha. Several anthropic installations were discovered from the Mesolithic to the Iron Age. 282 structures dated or attributed to the Mesolithic period have been found. For the Neolithic, 126 pits mainly "U-shaped, V, X and Y-shaped" (Schlitzgruben type) were excavated. These 408 pits are distributed in irregular lines over the entire surface of the excavation. For the Neolithic period, a late Neolithic burial (individual 1142) is located in the last phase of filling of a Schlitzgruben-type pit and a burial (Sep. 2309) dated from the Middle II-Late Neolithic transition contains the remains of two individuals.

21- Les Hautes Chanvières[14,15] (Mairy, Ardennes) – C. Laurelut

A very large site with a “Michelsberg” enclosure barring the confluence zone of the Meuse and Chiers, Mairy (region of Sedan, Ardennes) was excavated from 1981 to 1991. In addition to the ditch and the palisade system blocking the old confluence, the site presents about 50 long houses and about 200 "silo pits" containing very rich material deposits mainly ceramics and faunal remains. Mairy can be interpreted as a central site controlling a large area. It combines the functions of living area and ceremonial site, probably also a major economic center for this region, especially with regard to the pastoral economy. It shows multiple cultural long distance affinities, certainly favored by its position on a major north-south axis. On the Meuse, it is also located at a tipping point between the Rhine zone and the western area of “Michelsberg” in the eastern basin of the Seine. As it can be assessed on its basis of ceramics serialization, site phasing and absolute dating, the occupation covers several centuries in the first half of the 4th millennium.

Only three burials have been identified on the site, in association with the dam. Two burials, radiocarbon dated to around 4000 BCE would be associated with the foundation of the site, the last, dated to 3600-3500 BCE, to its abandonment.

22- Le Prieuré (Isle-sur-Marne, Marne) – O. Baillif

The excavation of Isle-sur-Marne Le Prieuré confirmed the presence of occupation since the Neolithic (hollow structures: pits in "V" or schlitzgruben and silos). This presence continues until the Late Bronze Age. Two burials, including one double, were found in a bilobed pit and a silo. Two other Neolithic burials were discovered within a contiguous plot.

In the silo two adult (young and mature) females were buried simultaneously, probably under similar perishable covers. The bodies are in a bent position on the left side, one behind the other. A sandstone bead was found near the cervical spine of subject 1. Despite fine sieving (1 mm mesh) of the sediment covering and around this anatomical area, no beads were found. Radiocarbon dating, carried out on the subject 2, made it possible to asign this double burial to the recent Neolithic.

23- Chemin dit des Royats (La Cheppe, Aube) – I. Richard

An archaeological survey was conducted in March 2009 at La Cheppe "Chemin dit des Royats" (Aube). Two distinct occupations were found, a collective grave from the late Neolithic and several Gallo-Roman structures.

An original, seemingly isolated, funerary structure radiocarbon dated to the Neolithic period was manually excavated. According to the observations made, the pit seems to have been made for the sole purpose of burying individuals. The remains of four individuals, whole or lacunary, were found, successively buried in varying positions. Following the first two burials - individuals with traces of voluntary or involuntary carbonization - a layer of a smoky sediment was found containing in addition to burned remains of human bones (fragments of charred individuals or the cremation of a fifth individual?), animal bones, some ceramics, charred stones and carpological remains.

The lower levels show signs of disturbance. Another individual was later deposited in several areas in the form of skeletal fragments. Finally, a fourth individual was in the upper level, in a complex position. The pit shows no sign of fire and the partial and atypical thermal alteration of some bodies (extremities in particular) probably took place in another place.

Two radiocarbon dates were made by the "Centrum voor IsotopenOnderzoek" in the Netherlands on a long bone fragment of the lower limbs of individuals 1 and 2. The results obtained give, for the individual 1: 4105 +/- 35 BP a date between 2870 and 2500 BCE with a precision order of two sigma;

for the individual 2: 4015 +/- 35 BP, which represents a dating between 2620 and 2460 BCE This information, thus expressed in two sigma, makes it possible to propose a dating within a range of 2800 and 2400 BCE of these two individuals at the very end of the late Neolithic.

24- La grotte Gazel (Sallèles-Cabardès, Aude) – J Guilaine

The Gazel cave in Sallèles-Cabardès (Aude) was frequented from the Upper Palaeolithic until the Middle Ages and includes a series of stratified occupations of the late Mesolithic and the early Neolithic. Over 1.80 m of stratigraphy, the deposits of the ancient Neolithic correspond to four stages (Gazel I to Gazel IV), the first correspond to Cardial, the other three to an original Languedoc Epicardial. Four epicardial burials have been identified, two complete and two partial.

25- Le Champ du Poste (Carcassonne, Aude) – F Convertini

The site of the Champ du Poste (Carcassonne, France) was excavated in 2005 and 2006. It is an important site for several archaeological periods that span from Neolithic to Antiquity. The Middle Neolithic is the period best represented. The duration of the occupations is known by a series of radiocarbon dates. During the first half of the 5th millennium BCE, the first settlers' installations leave few traces (heated stone hearths) and some burial pits. Then, during the Early Chasséen (second half of the 5th millennium BCE), occupations become more numerous and the site yields abundant artefacts (ceramics, flints, bones). Domestic structures (pits, silos, hearths) as well as burial pits are present. At the end of the Middle Neolithic, few structures and remains are present on the site. It was reoccupied at the end of the Neolithic (domestic structures and remains), then at the beginning and the end of the Bronze Age and finally again during Antiquity

26- Les Plots[16] (Berriac, Aude) – M. Gandelin

The site Les Plots in Berriac was excavated as part of rescue operations conducted between 1984 and 1991, under the direction of Jean Vaquer. On about 1 ha, 84 archeological installations were located on a mound that was probably an old terrace of the Aude. Most of these are hollow structures with a circular plan of the domestic type, the circulation levels are not conserved and the pits are cut off from their upper part. They testify to the existence of an ancient Chasseen settlement (dated between 4340 and 4230 BCE) established on a loess slick in a particularly fertile area. Among the discovered pits, six, scattered within the domestic space, contained individual primary burials. Both sexes are represented and children are also present. Items clearly associated with the deceased are rare although the filling of some pits include abundant fragmented ceramics. The observations made on Les Plots site do not reveal a strong homogeneity of funerary practices, except in the probable re-use of domestic graves for burials (Duday and Vaquer 2003).

27- Le Crès (Béziers, Hérault)[17] – M. Gandelin

The site of Crès in Béziers was excavated in 2001 by Inrap. This deposit was explored on an area of 5,000 m² that does not correspond to the entire archaeological site. Only 190 structures remain preserved and of these 30 yielded single or multiple primary burials. A total of 49 individuals have been discovered, which make it one of the most important burial complexes known for the southern Chasséen. This grave group is dated to the ancient Chasséen, between 4350 and 4100 BCE.

Some of the deceased, men or women, are installed in clear funerary graves, oblong plan and often arranged with large blocks of stones, while others are deposited, sometimes without particular care and most often without grave goods, in domestic pits, including silos. The latter type of pits can contain several individuals, including children who are frequently placed in multiple burials.

The grouping of burials testifies to a true spatial organization of the tombs with a separate burial area located close to the settlement.

28- Le Pirou (Valros, Hérault) – M. Gandelin

The Pirou site in Valros (Hérault) was excavated by an Inrap team in 2007. This hunting occupation, discovered after a 25,000 m² single-piece stripping, is installed on the southern slope of a small Miocene eminence. Up to more than 0.6 m of all the preserved structures are partially destroyed by taphonomic phenomena, mainly terracing, cultivation and erosion. All the facilities preserved are hollow structures, circular plan, interpreted as bases of pits or silos. Several have served as a place of deposit for individual primary burials. The anthropobiological analysis of the deceased shows that six adult female subjects and a girl buried on the site constitute, from the point of view of their biological identity, a heterogeneous group evoking the punctual burial of deceased from distinct groups. It should be noted, however, that exogamous marriages could also explain this variability.

The items collected from the site clearly testify to the domestic use of this installation of the ancient Chasséen but no element makes it possible to ensure the absolute synchronicity of the excavated pits or the permanent character of this occupation. The radiocarbon dates produced show a homogeneity that suggests an attendance that should not have exceeded four centuries and that, most probably, focuses on 150 years, between 4330 and 4260 BCE.

29- Villeneuve-Tolosane (Haute Garonne)

30- Cugnaux (Haute Garonne)

Since its discovery in 1945 by Louis Meroc, the Neolithic site of Villeneuve-Tolosane/Cugnaux (Haute-Garonne) has been the subject of a series of excavations that concerned more than a thousand archaeological structures spread over a total area about 50 ha. These various sites revealed traces of several successive hunting establishments consisting of pitted and/or palisaded enclosures with which are associated many domestic facilities (pits, heated hearths with pebbles, pole holes, etc.) as well as interhumations almost always in redevelopments (ditches, silos, pits). Seventeen of the eighteen documented burials are individual primary repositories—only one double deposit is attested—and are distributed throughout the site and contain both men and women, with children also well represented.

The chronology of these mortuary events is spread between 4400 and 3750 BCE. This data, correlated with the geographical dispersion of burials and the diversity of documented practices, do not encourage us to consider these deposits as belonging to the same burial complex: it is rather a succession of potentially independent manifestations of each other. Villeneuve-Tolosane and Cugnaux appear, after the studies that have been conducted, as a vast village having experienced several phases of occupation, three of which are clearly associated with fortification systems. The population of these villages was fully oriented towards an agro-pastoral production economy. Some individuals have been buried on the site but this practice does not seem to be the most common funeral practice since it concerns a very limited number of subjects.

31- Aven de la Boucle[18] (Corconne, Gard) - H. Duday

The aven de la Boucle at Corconne (Gard) is a natural cavity that served as a collective burial from the recent Neolithic, in the second half of the fourth millennium BCE. It continued into the late Neolithic (Ferrières culture), at the beginning of the third millennium. The total inhumations consist of roughly 75 individuals, with a marked selection according to the age of the deceased (massive exclusion of children). Complex layouts were highlighted, particularly at the level of access: a natural diaclase had been covered in the manner of a megalithic corridor, with a large removable horizontal slab equivalent to the "entrance cap" of covered walkways or dolmens; steps were intended to facilitate the descent of the bodies to the deep part of the cavity, located more than 10 m below the surface. This passage was condemned after its use for funerary purposes: a wall of slabs closed the inner part of the diaclase, the roof of the passage was tilted and the outer part of the diaclasis been completely filled with stones.

32- Le dolmen de Saint-Eugène (Laure-Minervois, Aude) – J Guilaine

The dolmen of Saint-Eugene, on the Russol domain in Laure-Minervois (Aude), consists of a megalithic tomb with a sub-trapezoidal plan, 15m long, inserted in a circular tumulus of 22 m in diameter. The dolmen itself is divided into a corridor, a long antechamber and a terminal chamber. The monument suffered a lot from the intervention of quarry workers. The chamber and antechamber were excavated between 1924 and 1928 by G. Sicard. They contained many anthropological remains discarded by the archaeologist and recovered later. The clearance of the tumulus, the excavation of the corridor and the forecourt took place between 1989 and 1994. Abundant artefacts include blades and arrows of flint, shale palettes, neolithic ceramics, campaniform vases, copper dagger, gold beads, diamond-shaped bronze, ebony, and numerous objects of parure. Radiocarbon dating indicates a construction of the monument in the second half of the fourth millennium BCE and its use throughout the third millennium BCE.

33- Le dolmen des Fades (Pépieux, Aude) – J Guilaine

The Dolmen des Fades in Pépieux (Aude), also called "Palet de Roland", is the longest dolmenic tomb in the South of France. It is a megalithic gallery of 24 m, excluding the tumulus. It is split into a corridor of 12 m, an antechamber and a room, both 6 m in length. The passage between these three parts was done through "oven doors". A victim of early disorganized excavations, a survey was carried out in 1946 by O. and J. Taffanel and J. Arnal in its terminal room, a sector that had in fact been disturbed in the Middle Ages. From 1962, J.Guilaine has excavated the corridor and the antechamber. The artefacts revealed a long use beginning in the late Neolithic and continuing until the Bronze Age. Several radiocarbon daes confirm this long occupation during the third millennium BCE.

34- Le dolmen des Peirières[18] (Villedubert, Aude) - H. Duday

The Peirières dolmen in Villedubert (Aude) has had a very special history. It was originally a medium-sized dolmen, the interior space being about 2 m wide by 12 m long. It seems to have been built in the late Neolithic, but only tiny fragments of the original funeral deposits remain. The monument was indeed deeply reworked in Chalcolithic. During the Bell Beaker period, most of the side jambs and the bedside of the room were removed or broken, and the hall and antechamber were completely destroyed. After removal of the remains of bodies, some still in process of decomposition, a "house of the dead" was erected on the same site, provided with a covering resting on two pillars of stones. This construction served as a collective burial for a relatively short period, and contained the bodies of nearly a hundred individuals, adults of both sexes and children of all ages. After which the superstructures of the tomb seem to have been disassembled, and the deposits were covered by a cope of large pebbles arranged with care.

35- Mitra 2 and 3 (Saint-Gilles-du-Gard, Gard) – B Sendra

The site of Mitra 3 has yielded three infant burials dated to the ancient Bronze Age.

At Mitra 2, (Saint-Gilles-du-Gard, Gard), four burial pits of the Late Bronze IIb were found; two with single burials and two others with multiple burials. There are a total of six individuals, one of which was radiocarbon dated, consisting of three adults, one adolescent and two children.

36- Quinquiris (Castelnaudary, Aude) – A. Alcantara

At Quinquiris (Castelnaudary, Aude), a lot of five sepulchral graves with burials; four are dated between the end of the Early Bronze Age and the beginning of the Middle Bronze Age to the last of the Late Bronze Age. In total, this deposit yielded 32 individuals of all ages.

37- Mas de Vignole IV (Nimes, Gard)

One pit dated from the late Bronze Age was found in Mas de Vignole IV (Nimes, Gard) containing two primary burials, both adults, one male.

38- Oppidum du Plan de la Tour[19,20] (Gailhan, Gard) – B. Dedet

The settlement of Plan de la Tour (Gailhan, Gard) is located in eastern Languedoc, in the foothills of the Cevennes. It was occupied between the fifth and fourth century BCE. The excavation delivered the burials of more than twenty subadults. These deceased were not incinerated, while cremation is the rule in this region for adults admitted to the village cemetery. They were buried in a small pit of the size of the body inside the houses. The bodies are not swaddled, as shown by the observation of the position of the limbs.

39- Oppidum de Pech Maho (Sigean, Aude) – E Gailledrat

Pech Maho (Sigean, Aude) is a small fortified trading post founded at the middle of the 6th century BCE and abandoned at the end of the 3rd century BCE. The settlement acted as a place of exchange and meeting between native populations and mediterranean merchants (Greek, Etruscans, Iberians). Domestic levels yielded some graves of very young children.

40- La necropole du Peyrou 2[20,21] (Agde, Herault) – B. Dedet

The Peyrou site at Agde (Hérault), was an incineration necropolis during the second half of the 7th century BCE (Peyrou 1) and yielded 35 burials which reveal very different funerary practices (Peyrou 2) which span the end of the 5th century to the middle of the 2nd century BCE. Among the subadults, there were 8 perinatals or infants buried in a vase. Adults were buried only with objects relating to the mortuary toilet (perfume vases) or a symbolic tribute with no difference according to the sex of the deceased. These practices are identical to the Greeks' rituals, very different from those of the surrounding region during the Iron Age. Ancient texts attest to the existence of a trade settlement created by the Greek colonists of Marseilles in this place at this time.

41- La Monedière (Bessan, Herault) – A Beylier

At La Monédière (Bessan, Hérault), a burial complex used in the course of the second century BCE yielded a small collection of burials.

The site of La Monédière is the place of a Gallic fortified settlement occupied between the early sixth century and the end of the fifth century BCE. Covering an area of nearly 4 ha, this settlement occupies a slight relief on the right bank of the Hérault river. Its foundation appears closely linked to very early contacts made in this part of the Gulf of Lyon with the Mediterranean societies and the establishement, at the mouth of the Herault, of the Agde littoral counter which is roughly 6 km away. The trading activities, indicated by the abundance of products imported from the Greek or Etruscan world, are generally at a level higher than that observed in neighboring establishments. The quantity of amphora reveal the involvement of this site in trade networks uniting the coast and the interior, as well as its role in the redistribution of the products transported from the Mediterranean within the framework of Mediterranean trade, by land or waterways. Benefiting from a favorable geographical position, La Monédière stands as an essential market place and a privileged meeting place between natives and Greeks, to such an extent that the question of the *in situ* installation of a community appears Hellenic. Its occupation is interrupted towards the end of the 5th century BCE at the moment of the founding of the colony of Agde/Agathè by Marseilles. In the course of the second century BCE, the site is revisited. At this time, a funeral complex yields a small collection of burials associated with a very poorly known settlement, which could be an integral part of the chôra of the Agathe colony.

42- Le Cailar[22] (Cailar, Gard)- R. Roure

The site of Le Cailar, south of Nîmes, is an important laguna harbour of the Iron Age. It is studied since the 2000s and archaeological excavations showed that the settlement is occupied since the 6th century BCE and very involved in the exchanges with Greek Marseille and all of the Mediterranean. The protohistoric and ancient occupation of Le Cailar lies at the confluence of Vistre and Rhôny. During the whole 3rd century BCE, many severed head and metal weapons were displayed on a large public place near the fortification. About 2700 fragments of human bones were recorded during ten excavation campaigns, almost all belonging to the skull. Chemical analyses proved those heads were embalmed

SI.2 Ancient DNA laboratory work

Processing of the samples systematically took place in a high containment laboratory located at the Institut Jacques Monod in Paris, where protective clothing and decontamination of reagents, surfaces and equipment was employed[23]. All the reaction buffers and enzymes used for the different steps of the library preparation were decontaminated beforehand using using UV-treatment of ethidium monoazide (EMA)[24]. Each mix was decontaminated just prior to use as follows: 0.25 volume of EMA 24 µM was added to the reaction mix in the dark, using a red light. Then the mixture was incubated for 10 minutes in the dark, prior to exposing the Eppendorf tube to a bright light for 6 minutes.

SI.2.1 Cutting and grinding

To maximize the recovery of DNA from long bones, we focused on the bone cortex, the dense bone located beneath the bone surface along the shaft. We first defined the sampling area by removing the outer layer of the bone with a clean razor blade, thus eliminating sediments and potential surface contaminants. In poorly preserved bones, we ground the underlying area into powder by low-speed drilling in order to avoid local heating that could damage DNA. Denser bones were cut into fragments with a diamond saw blade before performing cryogenic grinding using a freezer mill as a finer powder was shown to produce higher DNA yield.

Depending on the degree of preservation of the bone, the temporal bone was either part of a partially intact skull or separate. The particular structure we aimed at was the dense bone of the otic capsule which is encapsulated in a dense white bone [25]. From fragmented bones, it was exposed after scraping the outer bone layer with a razor blade and carefully removing the dense bone on the opposite side to the internal acoustic meatus through low-speed drilling. The cochlea was cut off the bone piece using a diamond saw blade, then powdered using a freezer mill. As complete skulls can sometimes be fragile or filled with compacted sediments, we found that the easiest and least damaging way to access the inner ear was to carefully drill it out of the ear canal.

The tooth root was bleached and sediments from its outermost surface was gently removed with a sterilized razor blade. A diamond saw blade was then used to separate the crown and the root, and as much pulp and dentine as possible was carefully drilled out of the latter as previously described[26]. The remaining outer layer of the roots was then used for the DNA extraction.

SI.2.2 **DNA extraction**

We opted for a DNA extraction at 37°C relying on Ethylenediaminetetraacetic acid (EDTA), a chelating agent that sequesters metal ions such as calcium cations (Ca^{2+}), therefore weakening the hydroxyapatite matrix that composes hard tissues such as bones. We coupled EDTA with a source of phosphate, in the form of disodium hydrogen phosphate (Na_2HPO_4) to compete with DNA and prevent it from binding back to the hydroxyapatite upon release. β-mercaptoethanol was added to the extraction buffer as it helps in denaturing proteins by breaking the disulfide bonds between the cysteine residues, but mainly as it was shown to remove the tannins and polyphenols present in crude extract during plant DNA extraction, and these are frequently co-extracted along with DNA during the procedure [27].

Over the course of the project we also opted for a two-step extraction procedure according to the rationale in Damgaard *et al.* 2015. Briefly, although colonization by microorganisms is likely to be heterogeneous within a bone [28], it is expected that during the digestion of bone material with EDTA, surface contaminants would be released into solution first. This also applies to modern human DNA contamination, which would be deposited on the bone surface during sample handling. Endogenous DNA, preserved within local "niches" [29], might be better protected and therefore released after further degradation of the bone matrix after a longer incubation time. Thus, treating the ground bone material with a digestion buffer for a short period of time (a "pre-digestion") would remove a fraction of the exogenous non-target DNA and thereby enrich the DNA extract for endogenous DNA [26]. The addition of a pre-digestion step to the protocol resulted in the use, for the first incubation period, of both a detergent, in the form of N-Lauroylsarcosine sodium salt (NLS), and a proteolytic enzyme (Proteinase K), as they respectively degrade cell structures (here expected to be those of microorganisms) and degrade the proteins that would be released as a result.

The three extraction buffers used were prepared in small batches prior to the extraction and UV irradiated, then each was processed along with samples as an extraction blank.

Between 100-250 mg of bone powder was incubated in 1 mL extraction buffer following a two-steps procedure: a 15 min incubation at 37°C in 0.25 M Na_2HPO_4, 10 mM EDTA, 50 mM Tris/HCl, 0.5% NLS, 1% β-mercaptoethanol and 250 µg/mL proteinase K, followed by removal of the supernatant and re-suspension of the pellet in 0.5 M EDTA, 0.25 M Na_2HPO_4, 1% β-mercaptoethanol, 50 µg/mL proteinase K for 48-72 h at 37°C. Teeth roots were incubated in 2 mL 0.5 M EDTA, 1% β-mercaptoethanol, 50 µg/mL proteinase K for 48 h at 37°C.

SI.2.3 **DNA purification**

We chose a single silica column-based DNA extraction method adapted from Dabney *et al.* 2013, which was a modification of a protocol optimized for ancient bones and teeth[30], widely used in ancient DNA studies[31]. Insoluble debris from either the second extraction of bones or the incubation of tooth roots were pelleted for 2 min at 13000 rpm, and DNA was purified from the collected supernatant using a method adapted from the QIAquick Gel Extraction purification kit protocol (Qiagen), developed in the lab. The modifications are as follows. A sample volume of 1 mL was combined with 6 mL of QG binding buffer and 4mL of isopropanol and bound to the silica column on a vacuum manifold. 20 mL tube extenders[32] (Qiagen), that had been submerged in bleach and rinsed with water, were fixed to the columns, allowing pouring a larger volume, thus limiting handling. An additional wash with 2mL of QG was added, prior to the PE washing step, whose volume was increased from 750 µL to 2mL. After washing, columns were removed from the manifold and placed back in their respective 2mL collection tube for 1.5 min centrifugation at 13000 rpm on a bench-top centrifuge (Eppendorf). After being dry-spun for another 1.5 min at 13000 rpm, the columns were placed in a new Eppendorf tube and DNA was eluted twice in 30 µL of TET buffer (EB buffer (Qiagen), 0.05% Tween 20) heated to 37°C.

SI.2.4 **Library construction**

Double-stranded libraries were prepared following the protocol described in Gorge et al 2016.

Adaptors were conceived from the P5 and P7 adaptors of the TruSeq DNA SamplePrep Kit Library quantification and amplification. The DNA concentration and size distribution in each library was measured by qPCR, with a Qubit 2.0 fluorimeter (Life Technologies) and on a Bioanalyzer (Agilent), respectively.

SI.2.5 **DNA enrichment**

Sequence enrichment from both the whole mitochondrial genome and nuclear regions was performed through a capture approach using biotinylated RNA baits obtained through the in vitro transcription of PCR amplified target regions[33] (Dataset S3).

SI.2.6 **Mitochondrial baits synthesis**

The capture of the human mitochondrial genome was adapted from the protocol used to capture bovine mitochondrial genomes in the laboratory[34]. Briefly, a total of 11 pairs of primers were designed using OLIGO6.67 that would generate as many overlapping PCR products, ranging from 1500 to 1800 bp, covering entirely 16.5 kb-long human mitochondrial genome (Dataset S3). Annealing temperature was homogenized across pairs to be around 60°C, and a T3 polymerase promoter was added upstream of either the forward or reverse primer of the pair, enabling us to transcribe and generate complementary probes for both strands. Each PCR reaction was performed in a final volume of 50 µL containing 4 ng of human genomic DNA, 1U of FastStart Taq, 1x FastStart buffer, 3 mM MgCL2, 200 µM dATP, dCTP, dGTP and dUTP, 1 mg/mL BSA, and 1µM of one of the 22 primer pairs (Dataset S3) PCRs were performed on an Eppendorf MasterCycler epGradientS as follows: polymerase activation at 95°C for 10 min, 45 amplification cycles (95°C for 20 sec, 58°C for 30 sec, 72°C for 3 min), and a final extension step at 72°C for 5 min. The amplification of each fragment was visualized by agarose gel electrophoresis in a 1% agarose gel (100 volts for 30 min), and the products were purified using the QIAquick PCR purification kit. The purified mitochondrial fragments were then quantified on a Nanodrop ND-1000 spectrophotometer. The products were pooled in equimolar amounts in two different lots: one containing the fragments with the T3 RNA polymerase promoter sequence at the 5′ end of the forward strands, and the other with fragments containing the T3 RNA polymerase promoter sequence at the 5′ end of the reverse strands.

SI.2.7 Nuclear baits synthesis

For nuclear markers, in order to be able to capture independently both DNA strands without the necessity of two primer pairs per marker, we used a two-step PCR approach to attach the T3 promoter at the 5' of either the forward or the reverse strand via a 5bp-long linker sequence that differs on both sides (Dataset S4**).**

The amplification of each marker was performed using qPCR on a LightCycler480 (Roche), in a 384-well plate. Two wells were dedicated to the sample amplification, and two other wells were negative controls where genomic DNA was replaced by nuclease free water. The PCR mix was as follows: LightCycler480 SYBRGreen I master 1x, 4 ng of a male genomic DNA, and nuclease free water up to 7 µL. The mix described above was distributed between the different wells using an Eppendorf robot, then 3 µL of a 1.7 µM primer dilution was added to the corresponding well. Cycling conditions were as follows: 8 minutes denaturation and Taq activation at 95°C, then 40 cycles of 15 seconds denaturation at 95°C followed by 45 seconds annealing and extension at 60°C. One microliter of each previously amplified product was transferred to a new Eppendorf tube to form a pool, and the volume was brought up to 100 µL to obtain a 1/100 dilution of each individual product. Then, each individual product was diluted up to 1/100 000 in the final pool. The re-amplification step brings the T3 promoter upstream of either the forward or the reverse strand. In each case, the other primer of the pair brings a custom sequence, carefully designed not to be complementary to the sequence of any of the Illumina adapters used to prepare the ancient DNA libraries and to be uncommon, if not absent, throughout the human genome. Initial denaturation and polymerase activation took place for 5 min at 95°C. The first five cycles of amplification consisted of 30 seconds denaturation, followed by 30 seconds annealing at 45°C and 60 seconds elongation at 60°C. Then for 10 cycles, annealing and elongation were performed together using a single 90 seconds step at 60°C.

In vitro transcription of the mitochondrial and nuclear pools, using as template the amplicons that harbor a T3 promoter upstream of either the forward strand or the reverse strand, took place at 37°C overnight. Four reactions were therefore set up as follows: T3 enzyme mix 1x, Transcription buffer 1x (Life Technologies), NTPs 0.5 μM each with a 1 to 1 ratio of biotin-14-CTP/CTP (NTPs and biotin-14-CTP Life Technologies), template DNA between 100 ng (nuclear fragments) and 500ng (mitochondrial fragments) and nuclease free water up to 40 μL. A the end of the transcription, this DNA template was digested by incubating the transcription reaction with 2 μL of TURBO DNase I for 60 minutes at 37°C. Transcription products were purified by performing a phenol-chloroform extraction followed by ethanol precipitation. The quantity of RNA was measured before and after purification with a Qubit 2.0 fluorimeter (Life Technologies). Fragmentation of the long mitochondrial fragments took place in a magnesium rich buffer at 94°C for 4 minutes (NEBNext Magnesium fragmentation module).

SI.2.8 **Blocking oligos synthesis**

Blocking oligos were obtained through the amplification of each N500- and N700-series barcoded sequencing adapters of the NexteraXT series (Illumina) with the T7 RNA polymerase promoter sequence at the 5′ end of the forward primer. (N500-series adaptor blocking oligos: 5′ATGTAATACGACTCACTATAGGGAATGATACGGCGACCAC3′ and 5′GGAAGAGCGTCGTGTAGG3′; N700-series adaptor blocking oligos 5′ATGTAATACGACTCACTATAGGGAGATCGGAAGAGCACACG3′ and 5′CAAGCAGAAGACGGCATAC3′. Each PCR was carried out in a 90 μL final volume reaction containing 1 nM of Nextera barcoded adapter, 1 U Faststart Taq DNA polymerase, 1x Faststart buffer, 90 μM of a mix of dNTPs, 1 mg/mL of BSA and 0.5 μM of N500- and N700-series adaptor blocking oligo primers. Cycling was performed on an Eppendorf MasterCycler epGradientS using a polymerase activation step at 95 °C for 10 min, followed by 25 amplification cycles (95 °C for 20 sec, 60 °C for 30 sec, 72 °C for 45 sec), and no final extension step. PCR products were visualized on a 2% agarose gel (100 volts for 30 min), and then quantified using a Qubit 2.0 Fluorimeter. Two equimolar pools were assembled from N700- and N500-series PCR products respectively, and purified using the Qiagen Gel extraction protocol and eluted with 50 μL of EB (Qiagen). Each purified pool was quantified using a Qubit 2.0 Fluorimeter, and 50 ng were used as a DNA template in a 40 μL MAXIscript T7 in vitro transcription reaction (Ambion) containing 1 mM of each NTP. After an overnight incubation at 37 °C, a 1 h TURBO DNase treatment was carried out at 37 °C to remove the remaining DNA template. The DNase, along with unincorporated nucleotides and buffer components, was removed through phenol-chloroform purification followed by ethanol precipitation. Purified transcripts were visualized on a 2% agarose gel (100 volts for 30 min), and quantified using a Nanodrop ND-1000 spectrophotometer.

SI.2.9 **Hybridization and hybrid pull down**

The capture was performed according to a published protocol [34], with subtle changes added over the course of the study. It consisted of either one or two steps of hybridization with PCR amplification in between. The hybridization of the biotinylated RNA baits to the DNA took place in an Eppendorf MasterCycler epGradientS set up to 62°C, and consisted of mixing the following reagents while keeping a small volume (around 25 to 30 µL): between 100 and 200 ng of input DNA library, denatured for 5 minutes at 95°C beforehand, an equivalent amount of RNA baits, a five times excess of blocking oligonucleotides to prevent unspecific hybridization of the baits to the library adapters, and half of the reaction volume of a 2x hybridization buffer prepared as follows: 10x Saline-Sodium Phosphate EDTA (SSPE) buffer, 10 mM EDTA, 0.2% Tween-20. Each reaction was then mixed by pipetting and incubated at 62°C for 48 h.

The capture of the RNA-DNA hybrids was performed using streptavidin-coated magnetic beads: either Dynabeads M-280 Streptavidin (Life Technologies) or Dynabeads® MyOne™ Streptavidin C1 ThermoFisher Scientific). 50/30 µL of beads per sample were washed three times in 1.5 mL of 1 M NaCl, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.01% Tween-20, then re-suspended in the previous buffer and Denhardt's solution (0.5x final concentration) and added to the hybridization mix and incubated 40 min at room temperature on a heatblock with constant agitation to prevent sedimentation of the beads at the bottom of the well. After the bead binding step, five post-hybridization washes were performed. The first two, lasting 15 and 10 min respectively, were performed at room temperature with 200 µL of 5x SSC, 0.1% Tween-20. The three remaining washes were performed with 200 µL 1x SSC, 0.1% Tween-20 for 5 min, the first one at room temperature, the last two washing steps at 62°C, using a pre-warmed washing buffer. For the second round of capture, the last washing step was performed using 0.5x SSC, 0.1% Tween-20 buffer for an increased stringency. To break the biotin-streptavidin bonds and release DNA fragments, the enriched libraries were eluted in EB buffer (Qiagen) complemented with 0.05% Tween-20 and heated at 95°C for 5 minutes, except for the earliest captured samples for which the elution was performed twice using 50 µL 0.1 M NaOH, and subsequent purification with QIAquick Gel Extraction kit (Qiagen) following manufacturer's instructions.

SI.3 Population clustering and data analysis

SI.3.1 Data collection and processing

Based on an initial screening of a total of 492 libraries covering 243 unique individuals, 246 libraries were captured for both the complete mitochondrial genome and sequenced on an Illumina MiSeq sequencer at the Institut Jacques Monod. Shotgun sequencing was performed on 58 separately built libraries using an Illumina NextSeq at the Institut de Recherche Biomédicale des Armées in Brétigny-sur-Orge.

Analyzing our newly generated and complex dataset required the assembly of a comparative dataset of ancient individuals sampled across western Eurasia. We gathered raw sequencing data from 647 ancient individuals from Iberia (96), Great-Britain (140), Central Europe (327), Anatolia (24) and Russia (31), and 21 published ancient French obtained through shotgun sequencing or targeted enrichment of 1.2 million SNPs, taking advantage of the complete mitochondrial genome capture that is performed alongside the latter. We also used genotyping data from the modern populations of the Human Origins panel[35].

Adapter sequences were trimmed from our newly generated sequences and overlapping paired-end reads were merged using LeeHom with the --ancientdna option [36]. Cutadapt was then used on the complete dataset to perform quality trimming with a threshold of 10 (-q 10), and subsequently filter out reads shorter than 30 bases (--minimum-length 30)[37].

Merged reads were also mapped to the human 1000 Genomes project build hs37d5 with decoy contigs using BWA version 1.2.3 [38], and against the revised Cambridge reference sequence (rCRS)[39] for human mitochondrial DNA with a duplication of its first 100 bases at the end to ensure mapping of the reads overlapping the junction resulting from the virtual linearization of the circular mitogenome.

SI.3.2 Contamination and authenticity

Sequences authenticity was estimated on libraries subjected to either a partial or no USER treatment using MapDamage [40]. Mitochondrial contamination was estimated after sequencing using schmutzi[36] (Dataset S5), while we used heterozygous transversion sites on the X chromosome of male samples to obtain an estimate of nuclear contamination for those samples using ANGSD[41]. This analysis excluded the pseudoautosomal region of the X chromosome and a minimum base and mapping quality threshold of 30 was applied while no coverage filter was used. We report the result of the ANGSD method 1 and 2 in Dataset S6.

Although we only used published data obtained from UNG treated libraries, traces of ancient DNA damage sometimes remain and manifest as a slighty higher frequency of C to T transitions on the end of each molecules. We trimmed 2 bases on both extremities for all partially-treated UDG libraries.

SI.3.3 Mitochondrial dataset analysis

3.3.1 Mitochondrial haplogroup determination

Consensus sequences were obtained using the software ANGSD v0.910, relying only on reads with base quality above 20, mapping quality above 30 and with more than 3x coverage. Mitochondrial haplotypes were determined based on the PhyloTree phylogenetic tree [42] build 17 and by using the HAPLOFIND web application [43], and Phy-Mer [44]. Unexpected or missing mutations were visually inspected in Geneious version R6 [45] to check if they could be results of misincorporations in low coverage regions. When the coverage was insufficient for reliable consensus calling, the BAM file was inspected visually in order to detect SNPs that were supported by reads with a total coverage of 3x. these were reported and used for haplogroup frequency bases approaches only. Individuals who did not reach sufficient coverage or resolution (with more than 5% undetermined bases on the mitochondrial genome), or were reported or assumed to be related to another individual in the dataset, sharing the same funerary structure/tomb and maternal lineage, were removed.

We here report a total of 223 new mitochondrial genomes from ancient French, 201 obtained through targeted enrichment and 22 through low coverage shotgun sequencing only (Dataset S5 and Dataset S6). This dataset encompasses Mesolithic (5), Neolithic (149), Bell Beaker (2) Bronze Age (36) and Iron Age (31) individuals sampled from across the Hauts-de-France (north), Grand-Est (east) and Occitanie (south).

3.3.2 Population clustering

We combined our 5 Mesolithic hunter-gatherers with published Holocene hunter-gatherers from Western Europe (after 10,000 BCE)[46–48]. The remaining dataset was split in seven time periods: namely the Early Neolithic (5300–4700 BCE), the Early Middle Neolithic (4700–4200 BCE), the Late Middle Neolithic (4200-3500 BCE), the Late Neolithic (3500-2500 BCE), the Bell-Beaker (2500-2200 BCE), the Bronze Age (2000-800 BCE) and the Iron Age (800-25 BCE). This temporal breakdown combined with geographical groups as shown in Figure S3-1 depending on the sampling gave rise to the following groups for our data: NFEN (LBK culture), EF-MN1 (Early Middle Neolithic, Eastern France), NF-Mich (Escalles, Pas-de-Calais), EF-Mich (Bergheim, Alsace), SF-MN (Chasseen, Southern France) for the Late Middle Neolithic, EF-Noy (Noyen culture, Eastern France), SF-LN (Late Neolithic/Chalcolithic, Southern France), and NF-LN (Late Neolithic, Northern France) for the Late Neolithic, SF-BB and NF-BB for the respectively southern and northern France Bell Beaker, F-BA for Bronze Age and F-IA for Iron Age (Dataset S1). As for published data, we split the 645 individuals according to geography: Anatolia, Iberia, Great Britain, Central Europe and the Pontic

Steppe. These geographical groups were then further split in the same time periods mentioned above. The date interval covered by individuals within these groups was approximately 300 years on average. Further sub clustering was performed for the Late Neolithic period and the transition towards the Bronze Age according to existing population structures highlighted by nuclear data, thus separating Late Neolithic (LN), Chalcolithic (CA) and Bell Beaker (BB) associated individuals in Central Europe. The clustering was as follows:

- Early Neolithic Iberia (IEN) and Central Europe (CEN), the latter comprising Danubian cultures from Hungary (Starcevo, Koros, ALPc, LBKT) and LBK from Germany.
- Middle Neolithic Great Britain (GMN), Iberia (IMN) and Central Europe, the latter divided in two groups: CEMN ("Early Middle Neolithic") and CLMN ("Late Middle Neolithic") comprising individuals from Hungary (Lengyel, Sopot, Tizsa, Vinca) and Germany (Rössen) respectively.
- Late Neolithic Central Europe (CLN) that comprises Late Neolithic individuals from Germany and Corded Ware associated individuals from Czech Republic.
- Late Neolithic/Chalcolithic Iberia (ILNCA) and Central Europe (CCA), the latter comprising individuals from Chalcolithic Hungary.
- Bell Beaker Great Britain (GBB), Iberia (IBB) and Central Europe (CBB), the latter comprising individuals from Czech Republic, Germany, Hungary, Poland, Switzerland and the Netherlands.
- Bronze Age Central Europe (CBA) comprising individuals from Czech Republic and Germany.

This dataset is complemented with Western Hunter-Gatherers (HG), Anatolian Early Neolithic Farmers (ANE) and Bronze Age groups from the Pontic Steppe (SBA).

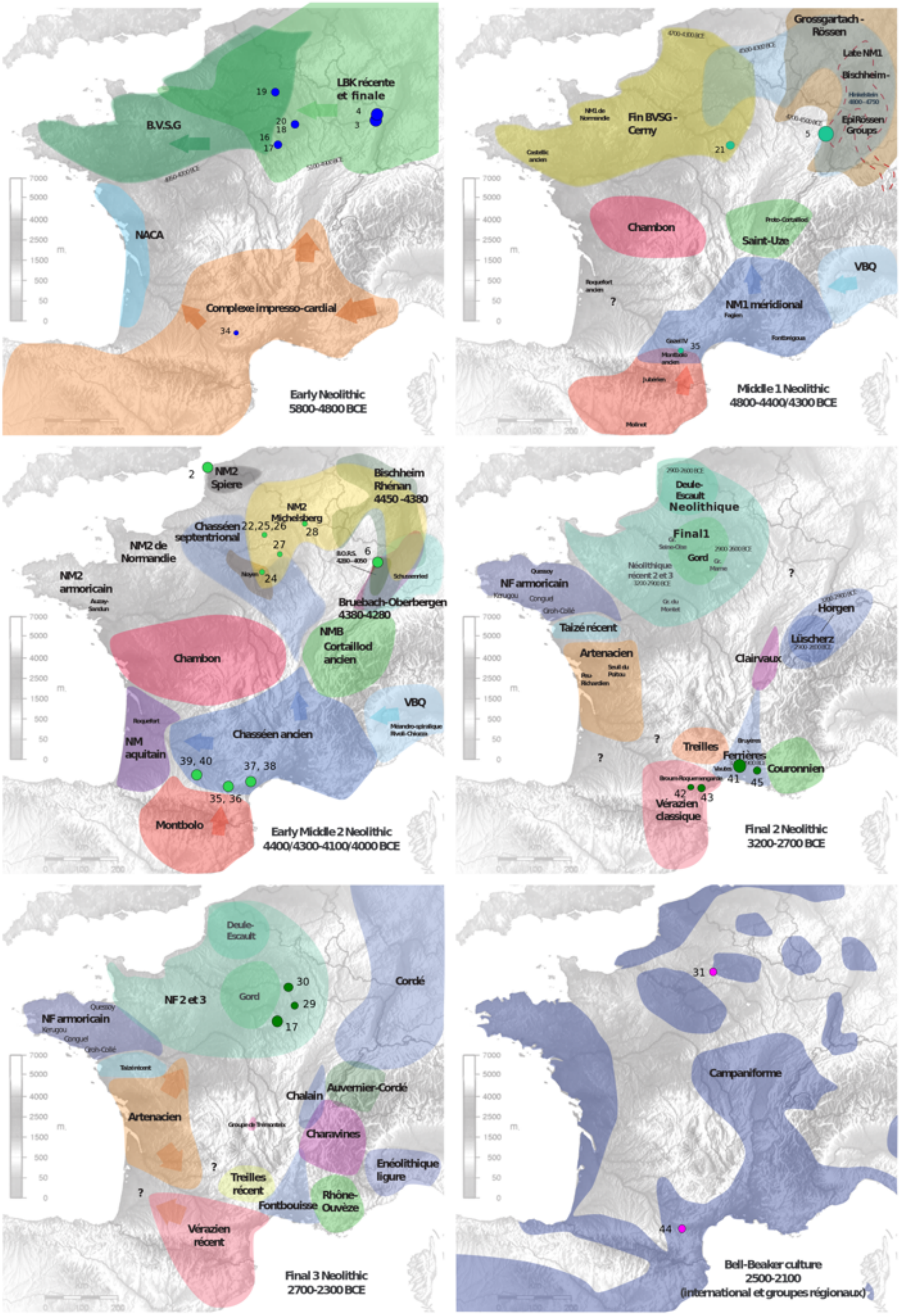


Figure S3-1: Map of archaeological culture from the Neolithic to the Bell Beaker and localisation of the samples studied (from M. Gandelin, F. Convertini, B. Perrin, J. Dubouloz, V. Ard, J. Vaquer, J. Guilaine) 1- La Grotte des Perrats (Agris, Charente) 2- Escalles (Mont d'Hubert, Pas-de-Calais) 3- Les terrasses de la Zorn

(Schwindratzheim, Bas-Rhin) 4- Ungeheuer Hoelzle (Morschwiller-Le-Bas, Haut-Rhin) 5-Rosheim Mittelfeld (Rosheim, Bas-Rhin) 6- Saulager (Bergheim, Haut-Rhin) 7- Innere Allmende (Niederhergheim , Haut-Rhin) 8- Muelhaecker (Bischwihr, Haut-Rhin) 9- Parc d'activités économiques intercommunal (Obernai, Bas-Rhin) 10- ZAC du Petit Prince (Rixheim, Haut-Rhin) 11- Buerckelmatt (Nordhouse, Bas-Rhin) 12- Untergasse (Erstein, Bas-Rhin) 13- Jardin des Aubépines (Colmar, Haut-Rhin) 14- Jebsenboden (Sainte-Croix-en Plaine, Haut-Rhin) 15- Ricoh (Wettolsheim, Haut-Rhin) 16- Saint-Léger-près-Troyes17- Les Pointes et les Grèvottes "ZAC St Martin 1" (Breviandes, Aube) 18- Les Noues, Journée Carrée (Orcontes, Marne) 19- Derrière le Village (Menneville, Aisne) 20- Le Champ Buchotte (Larzicourt, Marne) 21- (Buchères, Aube) 22- La Plaine (Beaurieux, Aisne) 23- Le Haut de Launois, Ferme de l'île (Pont-sur-Seine, Aube) 24- Pré Chevalier (La Villeneuve au chatelot, Aube) 25- Les Fontinettes, Champ Tortu (Cuiry-lès-Chaudardes, Aisne) 26- la Croix-Maigret (Berry-au-Bac, Aisne) 27- Recy (Châlons-en-Champagne, Aube) 28- Les Hautes Chanvières (Mairy, Ardennes) 29- Le Prieuré (Isle-sur-Marne, Marne) 30- Chemin dit des Royats (La Cheppe, Aube) 31- La Bouche à Vesle 32- Attichy 33- Bucy le long 34- La grotte Gazel (Sallèles-Cabardès, Aude) 35- Le Champ du Poste (Carcassonne, Aude) 36- Les Plots (Berriac, Aude) 37- Le Crès (Béziers, Hérault) 38- Le Pirou (Valros, Hérault) 39-Villeneuve-Tolosane (Haute Garonne) 40- Cugnaux (Haute Garonne) 41- Aven de la Boucle (Corconne, Gard) 42- Le dolmen de Saint-Eugène (Laure-Minervois, Aude) 43- Le dolmen des Fades (Pépieux, Aude) 44- Le dolmen des Peirières (Villedubert, Aude) 45- Mitra 2 and 3 (Saint-Gilles-du-Gard, Gard) 46- Quinquiris (Castelnaudary, Aude) 47- Mas de Vignole IV (Nimes, Gard) 48- Manduel 49- Le Cailar (Cailar, Gard) 50- Oppidum du Plan de la Tour (Gailhan, Gard) 51- Oppidum de Pech Maho (Sigean, Aude) 52- La necropole du Peyrou 2 (Agde, Herault) 53- La Monedière (Bessan, Herault)

3.3.3 Haplogroup frequency-based tests

To identify clusters among prehistoric populations with similar haplogroup composition, the frequencies of 20 haplogroups that were found both in our dataset and in the ancient comparative dataset were estimated (Figure S3-2).

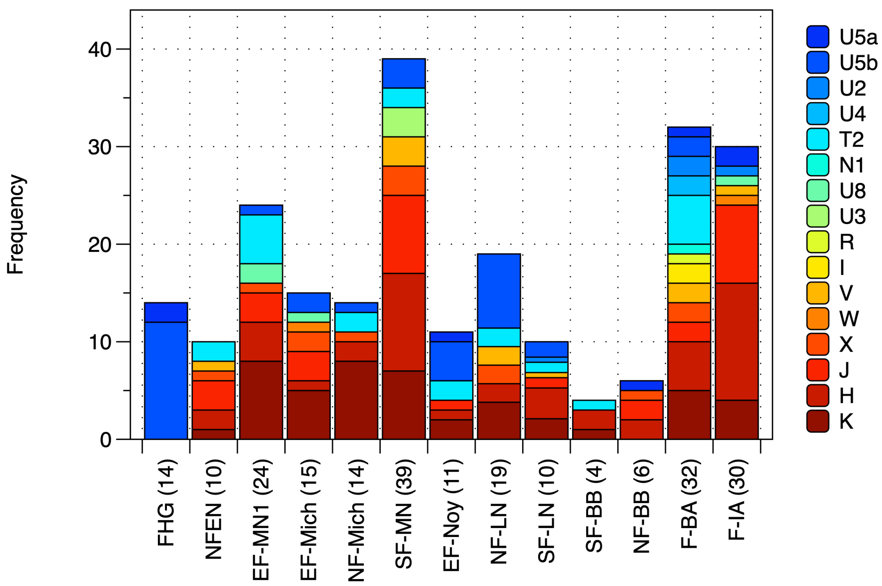


Figure S 3-2: Changes in the average frequency of core mitochondrial haplogroups from the Mesolithic to the Iron Age.

To unravel groups with similar haplogroup composition, we performed cluster analysis with the different French groups and all comparative datasets based on the haplogroup frequencies of the corresponding datasets. Principal component analysis was performed in PAST (http://palaeo-electronica.org/2001_1/past/issue1_01.htm), plotted in R 3.3.3. To assess the probability that the different groups are drawn from the same metapopulation, a statistical significance test, the Fisher's exact test was performed on absolute frequencies, using the package fisher.test in R 3.3.3[49] on the complete series of pairwise populations (Dataset S7).

We ran a set of descriptive statistical analyses on the combined European dataset at the haplogroup frequency level (using PCA, Figure S3-3) and at the sequence level (F_{ST}, Dataset S8) to study the distribution of maternal lineages in various European populations through time.

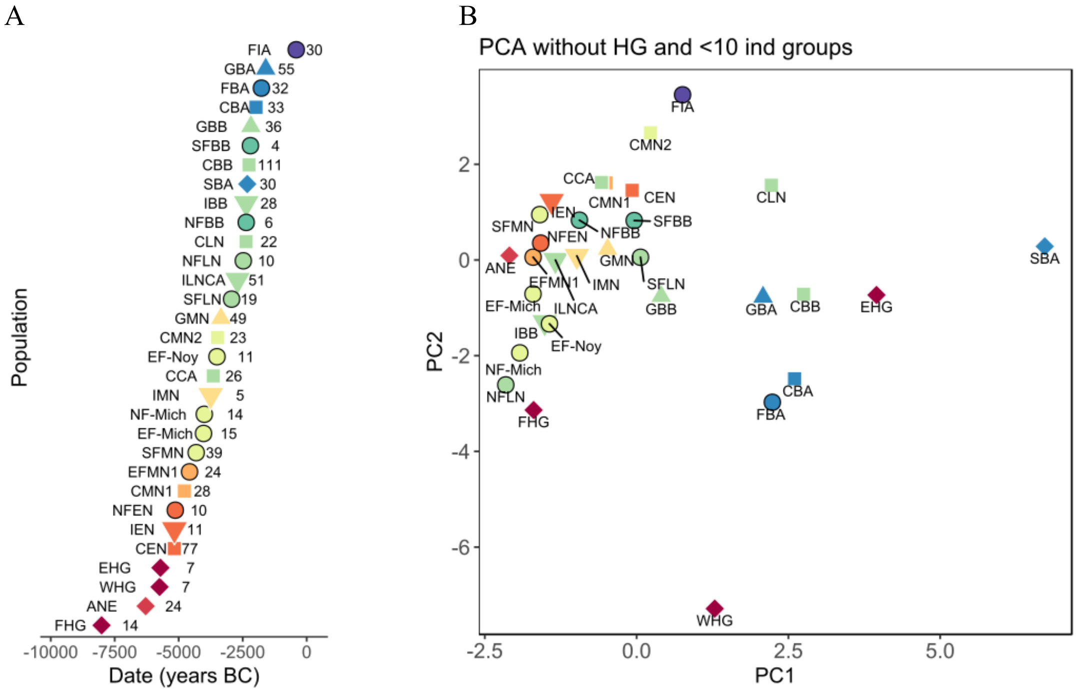


Figure S 3-3: Clustering of the individuals included in the present study by mitochondrial haplogroup frequency.

A. Dates (in years BCE) of the different populations and their respective number of individuals: FHG: French *Hunter-gatherers*, ANE: *Anatolia Neolithic*, WHG: *Western Hunter-gatherers,* EHG: *Eastern Hunter-gatherers,* CEN: *Central Europe Early Neolithic*, IEN*: Iberia Early Neolithic*, NFEN: *North France Early Neolithic*, CMN1: *Central Europe Early Middle Neolithic*, EFMN1: *Eastern France Early Middle Neolithic*, SF-MN: *South France Middle Neolithic*, EF-Mich*: Late Middle Neolithic (Alsace)*, NF-Mich: *Escalles (Late Middle Neolithic, Pas-de-Calais)*, IMN: *Iberia Middle Neolithic,* CCA: *Central Europe Chalcolithic*, EF-Noy: *Late Middle Neolithic (Champagne)*, CMN2: *Central Europe Late Middle Neolithic*, GMN: *Great Britain Middle Neolithic*, SFLN: *South France Late Neolithic*, ILNCA: *Iberia Late Neolithic/Chalcolithic*, NFLN: *North France Late Neolithic*, CLN: *Central Europe Late Neolithic* (Corded-Ware), NFBB: *North France Bell Beaker* SFBB: *South France Bell Beaker* , IBB: *Iberia Bell Beaker*, SBA: *Steppe Bronze Age*, CBB: *Central Europe Bell Beaker*, GBB: *Great Britain Bell Beaker*, CBA: *Central Europe Bronze Age*, F-BA: *France Bronze Age*, GBA: *Great Britain Bronze Age*, F-IA: *France Iron Age*. More information about the different populations and their clustering can be found in **Table S3-1 and 2. B**. Principal component analysis conducted on mitochondrial haplogroup frequencies. The two first components represent 22.5 % and 13.2% respectively of the total variance.

The first two components of the PCA, describing 35.7% of the total variability, reveal that all included populations fit within the edges of a triangle defined by Western Hunter-Gatherers (WHG), Anatolian

Neolithic farmers (ANE) and Bronze Age steppe pastoralists (SBA), with most Neolithic groups falling on a cline between ANE and SBA with little spread towards the HG edge.

The Mesolithic substrate (~ 11,000-7000 BCE)

We report the complete mitochondrial sequences of five new French HGs. Their analysis together with 15 individuals previously published[46,47] shows that the French Mesolithic substrate between 11,000-7000 BCE, was characterized by mitochondrial haplogroups U5b (85%) and U5a (15%), comprising U5b1, U5b1b, U5b1h, U5b1a, U5b2a, U5b2b and U5a2, already observed in European Mesolithic hunter-gatherers[46,50,51]. French Mesolithic hunter-gatherers were also characterized by a low nucleotide diversity (π 0.000923 +/- 0.000475), suggesting a rather homogeneous population across the territory.

3.3.4 Evolution of the populations during the Neolithic (~ 5500–2500 BCE)

The French Neolithic farmers (from ~5500 BCE onwards) display a wide diversity of haplogroups (U8, U3, N1a, K, J, T2, H, HV, X, V, W), with differences in frequencies depending on the period or the archeological site. Reinforced by the Fisher's exact test p-values, showing significant differences between HGs and every other population (Dataset S7) this disparity confirms the Anatolian ancestry of early Neolithic farmers in France, as elsewhere in Europe. HG mitochondrial haplogroups (*e.g.* U5b), however, are carried by individuals from the Middle Neolithic onward, indicating that some autochthonous hunter-gatherer maternal lineages were incorporated into farming communities over the course of the Neolithic. (Figure S3-2).

Data are very scarce for the earliest part of the Neolithic, corresponding respectively to the LBK culture that emerged from the Danubian LBK and became established in the Northern part of France, and to the Impressa/Cardial complex in the Southeastern part of France at the Mediterranean coast and further north into the Rhone valley. In total, we obtained ten reliable mitochondrial genomes (i.e. Phy-Mer score above 0.95[44]. from individuals from both Alsace and Champagne (Grand Est), and the Vallée de l'Aisne (Hauts-de-France) belonging to the LBK culture. We complemented these haplogroups with those obtained through the shotgun sequencing of two other individuals from Alsace (Mor6 and Schw432). Interestingly, the currently rare haplogroup N1a that was previously described as a signature haplogroup among the LBK in Central Europe [52], could not be detected in our samples. We estimated that, due to sample size, any haplogroup below 24% in the population would have a 95% chance of being missed, which is more than the frequencies of 15% and 10%, respectively, observed for N1a in Early Neolithic individuals from Germany and Hungary.

The Grossgartach (ca. 4700-4500 BCE) site of Rosheim (EFMN1) in Alsace, retains ties to Central Europe Early and Middle Neolithic (CEN, CEMN), as reflected by both their proximity on the PCA and their low pairwise F_{ST} values (Dataset S8).

Although there are no data available for the Early Neolithic in Southern France, both haplogroup frequency and sequence-based approaches agree on a genetic continuity between the populations of the French Middle Neolithic (SF-MN), belonging to the widespread Chasséen (~4400-3600 BCE) culture, and those of the Late Neolithic in both France (SF-LN) and Iberia (ILNCA).

In Northern France, however, the maternal make-up of archeological sites attributed to the second part of the Middle Neolithic (~4200-3500 BCE) is heterogeneous. It is notable that none of the three groups, namely from the West to the East NF-Mich (Escalles, Pas-de-Calais), EF-Mich (Bergheim, Alsace), are close to one another on the PCA (Figure S3-3). Escalles holds an outlier position due to a skewed distribution of haplogroup frequencies where one maternal lineage (K1a) constitutes more than half of the haplogroups (8/14 individuals). In contrast, individuals from the Michelsberg culture sampled in northern France (Mch) hold a particular position on the PCA and Ward's Clustering analyses, where they are closer to HGs than any other population. This affinity is linked to the high proportion of the mitochondrial haplogroup U5b (36%), a major maternal lineage in European Mesolithic HGs (19/29), indicating a particularly high maternal contribution of HGs in this region. This pattern is also visible in Late Neolithic individuals from the sampled regions, where 40% of mitochondrial lineages are from the U5b lineage. The site of Bergheim (~ 4300-3700 BCE, Alsace) is located 40 km from Rosheim, and is associated with the B.O.R.S (Bischeim occidental du Rhin Supérieur) and Michelsberg cultures. No significant difference is detected from both haplogroup frequency-based (Fisher's exact test p-value: 0.364) and sequence-based (F_{ST}=0.02, p-value =0.18424+-0.0039) approaches between the two populations. However, the maternal composition of the Bergheim group is responsible for its closeness to the population of Neolithic Great Britain (GBN).

As the Neolithic unfolds, we can observe in the French population a steady increase in the HG haplogroups, namely U5b and, to a lesser extent, U5a. Evident in particular from the second half of the Middle Neolithic (~4500 years BCE), this significant increase is not homogenous across the territory, which raises the question of the origin of these maternal lineages in the Neolithic background. In Champagne (Grand-Est) for example, the frequency of U5b haplogroups reaches 35% by the end of the 4th millennium BCE. At one archeological site in particular (Pont-sur-Seine), four out of five individuals sampled at random across the site belong to the U5 family and all fall within different haplogroups (U5b1h, U5b2c, U5b3b and U5a2d).

3.3.5 Transitioning into Bronze Age (~2300-1000 BCE)

We performed capture on 30 Bronze Age individuals across the French territory, to whom we added one individual who was sequenced at low coverage (RIX2). Since no geographical or temporal stratification was detected from the analysis of partial genomes from this period (Figure 1B), the 31 individuals were grouped together under the label F-BA. During the French Bronze Age, we observed the appearance of new mitochondrial haplogroups such as U2, U4 and I. These haplogroups were described as part of the «Steppe component» in Central Europe [53]. Thus, the situation on the French territory seemingly differs from the one in Iberia, where no major maternal impact from the population of the Pontic steppes has been observed [54–56].

3.3.6 Iron Age France (~ 800 – 100 BCE)

All French Iron Age individuals were grouped together as F-IA, independently of their geographical origin. We captured 14 individuals to whom we added 17 mitochondrial genomes obtained from the newly generated low coverage genomes. F-IA is located at the edge of the haplogroup frequency-based PCA (Figure S3-3). This position stems from an elevated frequency of maternal lineages H and J, reaching respectively 32.3% and 25.8% of the total haplogroups in this population. A comparatively high proportion of haplogroup H is only found in Bronze Age populations from the Pontic Steppe (SBA) where it reaches 30%, while J is frequent in Late Neolithic populations from Central Europe (CLN) and Iberia (ILNCA), where it reaches 22%. While Fisher's exact test indicates that F-IA is significantly different from Late Neolithic/Bell Beaker Iberia (pvalues: 0.044 and 0.0002), North France Late Neolithic (0.011), French Bronze Age (0.022) and British Bronze Age (0.027) (Dataset S7), pairwise F_{ST} values between F-IA and these populations are low and not statistically supported (Dataset S8). It is noteworthy though that fixation indexes were estimated from fewer sequences than haplogroup frequencies (14 instead of 31), and that F_{ST} is sensitive to sample size.

SI.4 Shotgun dataset analysis

Genotypes were called for the 1.2 million SNPs broadly used in aDNA in-solution capture procedures[57] A single allele was drawn at random for each position (minimum mapping and base quality of 30) using PileupCaller (https://github.com/stschiff/sequenceTools), rendering the individuals from the dataset homozygous for each locus. For genotyping data from modern individuals of the Human Origins panel, heterozygous sites in the eigenstrat file were randomly recoded as homozygous for the reference or the alternative allele using an awk script. The two files were then merged using plink v1.9[58].

We here report the genomes of 58 ancient French individuals, sequenced at coverages ranging from 0.042x to 0.576x. Samples are distributed across the territory and along the studied time transect, with 3 Mesolithic hunter-gatherers, 21 Neolithic individuals among whom 2 are associated with the Late Neolithic Bell Beaker cultural complex, 15 Bronze Age and 19 Iron Age individuals.

4.1.1 Principal component analysis

After mildly filtering the dataset for genotyping rate and linkage disequilibrium with plink v1.9 using the parameters –indep-pairwise 200 25 0.7, --geno 0.95 and –mind 0.95[58], principal component analysis was conducted where ancient individuals were projected onto the first 2 components of the PCA defined by modern Western Eurasian (lsqproject option from the smartpca package, Figure S 4-1). The modern day populations from the Human Origins data set used here are: French, Druze, Sardinian, Palestinian, Orcadian, Russian, Italian_North, Basque, Adygei, Saami_WGA, Bulgarian, Hungarian, Lithuanian, Iranian, Syrian, Lebanese, Jordanian, Saudi, Balkar, Georgian, North_Ossetian, Chechen, Abkhasian, Armenian, Lezgin, Nogai, Kumyk, Belarusian, Ukrainian, Estonian, Mordovian, Czech, Icelandic, Greek, Scottish, English, Spanish, Spanish_North, Finnish, Canary_Islander, Croatian, Norwegian, Sicilian, Italian_South, Turkish, Albanian, Cypriot, Lebanese_Christian, Lebanese_Muslim, Iranian_Bandari, Romanian and Assyrian.

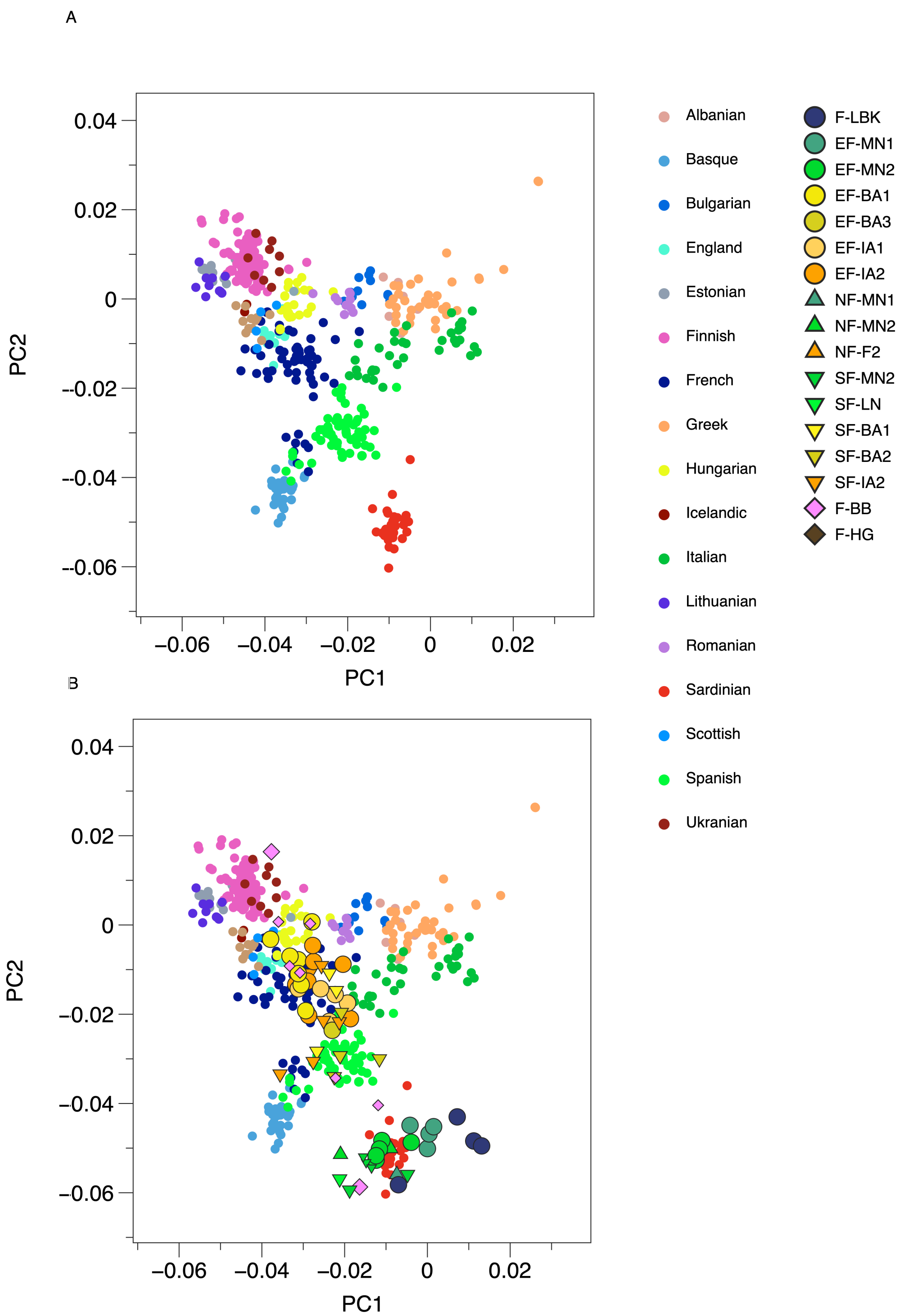


Figure S 4-1: Principal component analysis performed on present-day western Eurasians.

4.1.2 qpAdm

We use qpAdm (https://github.com/DReichLab) to estimate the ancestry of ancient French populations as a mixture of other ancient populations. This method uses f4 statistics in a regression context and incorporates methods from qpWave (https://github.com/DReichLab). It was developed by Patterson[59] and a more detailed explanation of this methodology is described in the supplementary information section 10 of Haak and colleagues[53]. The software tests a set of ancestral populations, differentially related to a set of outgroup populations, to define the proportion of admixture that contributed to our population of interest. It calculates a matrix of f4 statistics and formal p-values to determine whether the model is a good fit or not. We follow a similar strategy than previously described[53] and consider models with p-Value >0.05. The results of these tests are detailed in Dataset S9.

Mesolithic Period

We tested a model with the two population sources the Villabruna individual (Italy, 14000 cal BCE) and the GoyetQ-2 individual (Belgium, 15,230-14,780 BP) with respect to the following set outgroups[54]: Ethiopia_4500BP, Hungary_Koros_EN_HG, Ust_Ishim, Belgium_GoyetQ116_1, Russia_MA1_HG, Israel_Raqefet_M_Natufian, Papuan, Onge, Han, Karitiana, Mbuti.

Neolithic period and Bell Beaker period

We added Anatolia-N to our population source as a possible ancestry for the Neolithic population in France. We kept both Hunter-Gatherer sources GoyetQ-2 like and Villabruna like.

We also tested a 3-way and 4-way model by adding Russian Yamnaya Samara populations to our population source in order to estimate the arrival of Steppe ancestry in our late Neolithic and Bell Beaker individuals.

We investigated whether we could distinguish ancestry from cardial and LBK origin in our Neolithic population by testing 3-way model with our French Mesolithic individuals, Germany_LBK_EN and Iberia_EN as source populations but all models had a very poor fit probably due to the difficulty to distinguish cardial and LBK ancestry.

All samples could be successfully modeled as a 2-way or 3 way model with an Anatolian composant and one or two hunter-gatherer ancestries except one sample Schw75-12 (LBK) for which the best model is a 2-way model between Anatolian ancestry and Steppe ancestry (p-value 0.399). The Steppe ancestry highlighted in Schw75-12 can be interpretated as an eastern Hunter Gatherer component still present in the Yamanya population.

Bronze and Iron Ages

We first model Bronze and Iron Ages samples with the three source populations: Anatolian-N, the Villabruna individual and Russia Yamnaya Samara. All samples could be explained as mixtures of these 3 populations. We eventually tested a 4-way model by adding the GoyetQ-2 individual to test the survival of this composant in our populations. In all samples except samples from Bronze Age in the South of France, the 3-way model with Villabruna was a better fit.

We then tested if we could model Bronze and Iron Age populations as a mixture from previous French populations using the Middle Neolithic from the different regions and a French Bell Beaker CBV95 showing a high proportion of steppe ancestry as population source. In this case, we added Anatolia-N, Italy_Villabruna, Iberia_ElMiron, Germany_LBK_EN, HG-F, Iberia_EN, French-LBK and EF-MN1 in the outgroup reference. All samples could be modeled as a mixture of these 2 populations with good p-values.

None of our Iron Age samples shows evidence of Greek or Roman ancestry in our dataset.

4.1.3 **f3-statistics**

We used f3-statistics as introduced by Patterson[59] to measure shared drift among ancient individuals and populations relative to an outgroup[60]. This is commonly used to test whether a target population A is the result of the admixture of the two others. If A is chosen as an outgroup population (African Mbuti hunter-gatherers in our case), the value of the statistics when different from zero instead reflects the length of shared drift between populations B and C. By setting B as each of our ancient individuals/populations and C to all other modern and ancient populations from the literature, the magnitude of the f3-statistics indicates which populations are closer to one another. In order to assess significance, standard errors were computed using weighted blocks of jackknife over 5Mb blocks to compensate for heterogenous coverage of the different positions. To estimate how many standard errors the statistics deviates from 0, Z-scores are calculated, with statistical support for values strictly above 3 (or below -3).

To better appreciate the affinity of our ancient French to other ancient and modern populations, we proceeded to compute outgroup f3-statistics of the form f3(Ancient French population/individual, modern/ancient Western Eurasian population; Mbuti) (Figure S4-2, Figure S4-3).

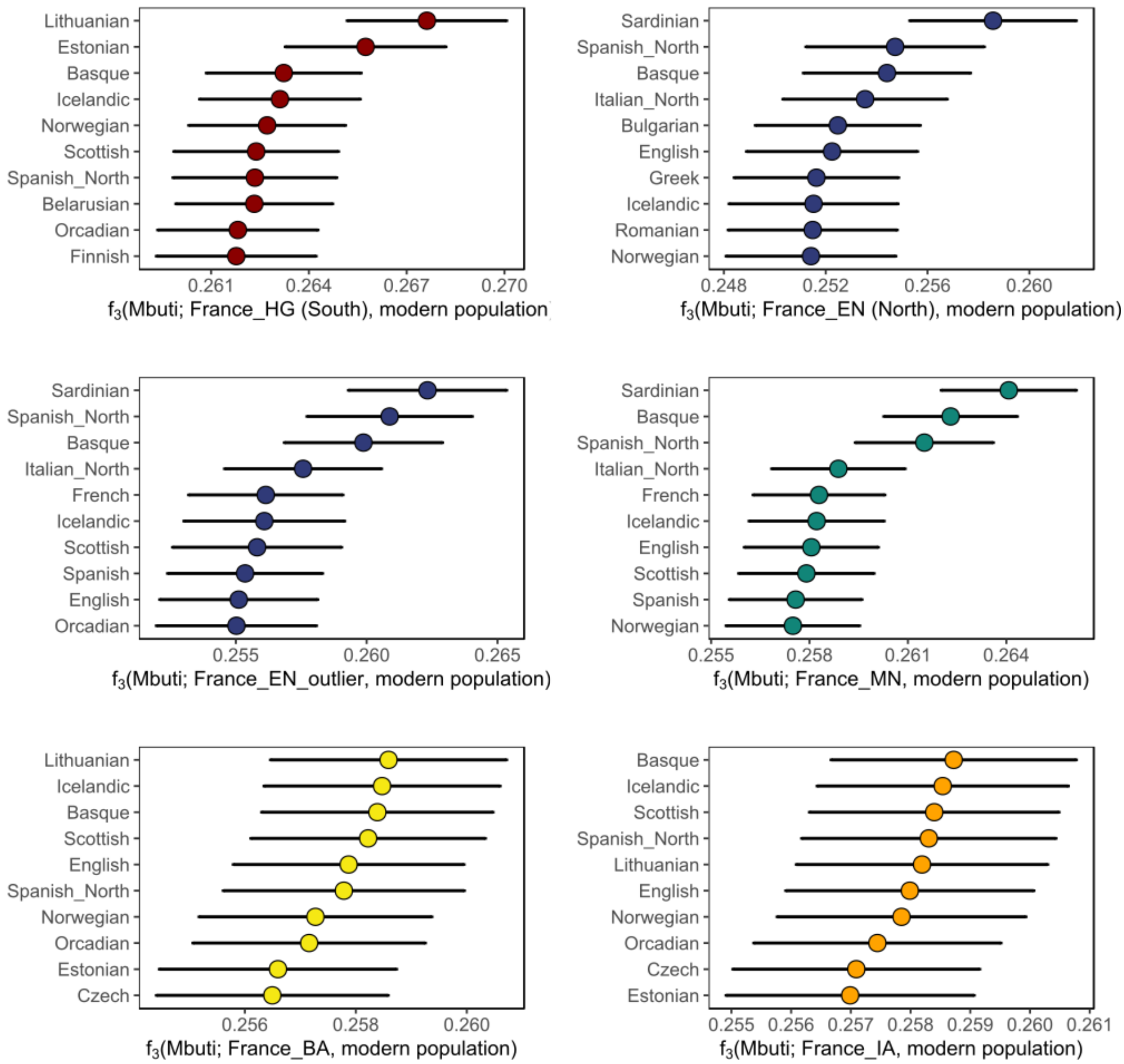
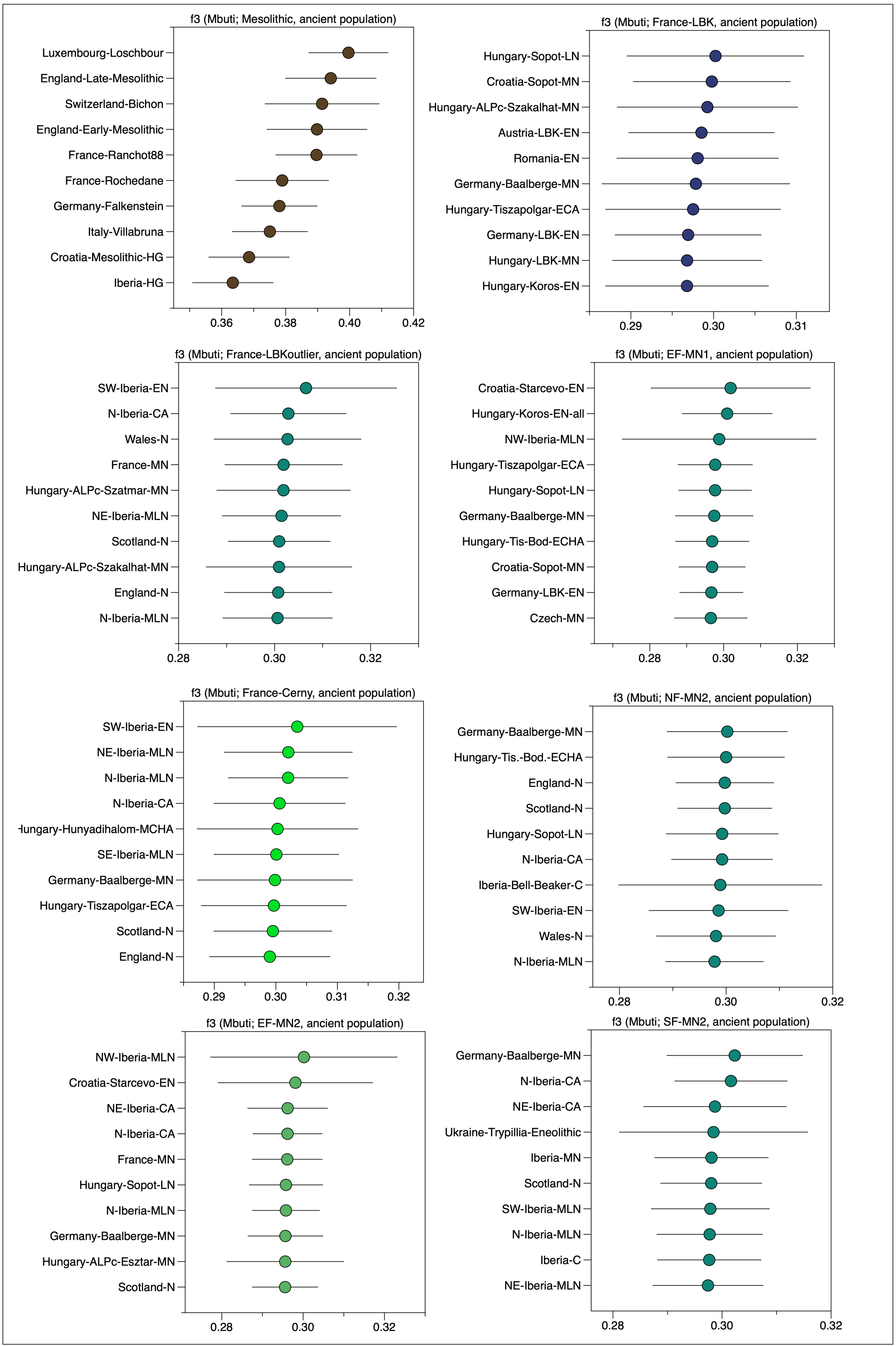
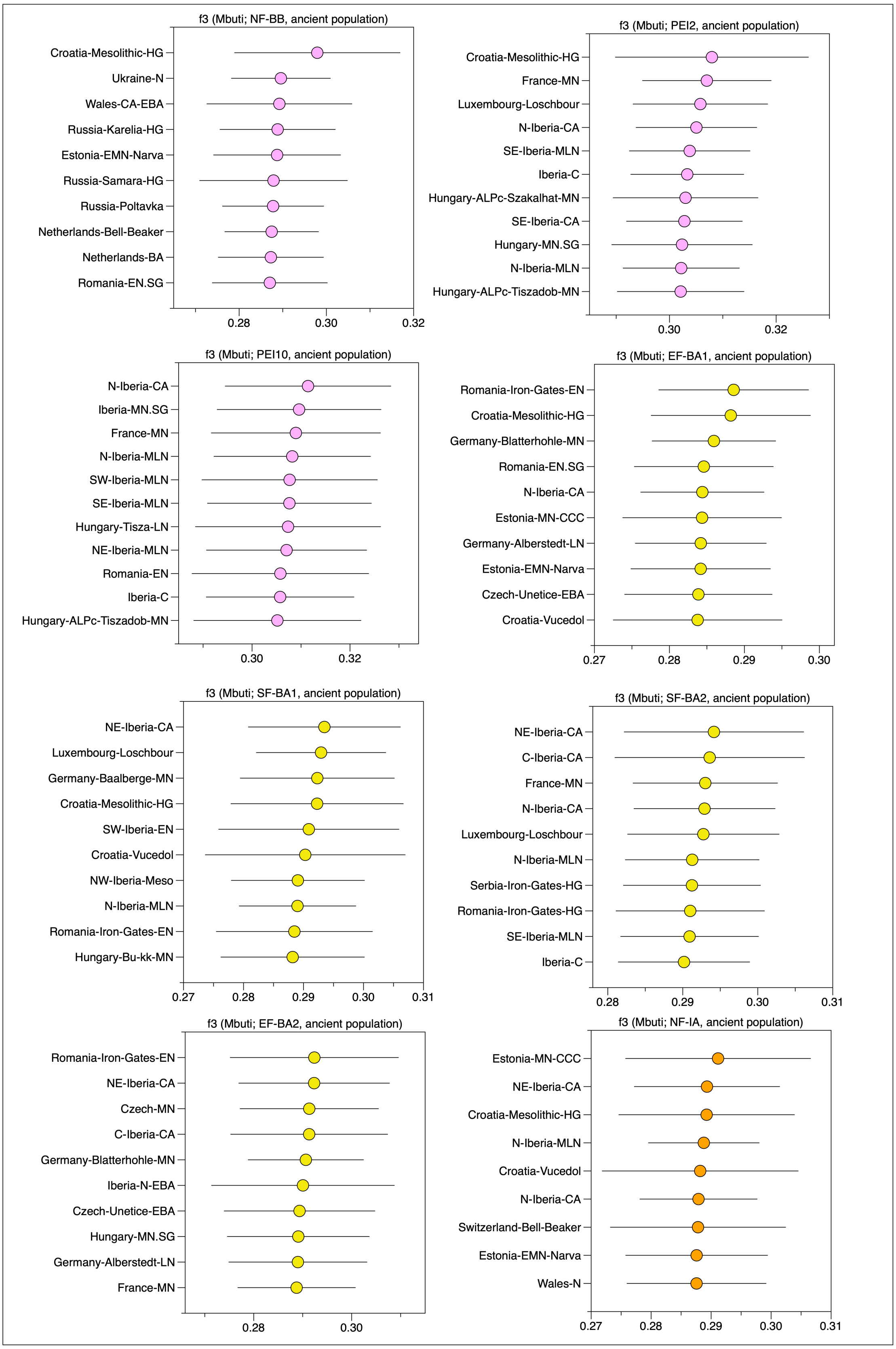


Figure S 4-2: Values of f3(Mbuti; ancient French individual, modern population) in ancient French populations.



f3 (Mbuti; Mesolithic, ancient population)
Luxembourg-Loschbour
England-Late-Mesolithic
Switzerland-Bichon
England-Early-Mesolithic
France-Ranchot88
France-Rochedane
Germany-Falkenstein
Italy-Villabruna
Croatia-Mesolithic-HG
Iberia-HG
0.36
0.38
0.40
0.42
f3 (Mbuti; France-LBK, ancient population)
Hungary-Sopot-LN
Croatia-Sopot-MN
Hungary-ALPc-Szakalhat-MN
Austria-LBK-EN
Romania-EN
Germany-Baalberge-MN
Hungary-Tiszapolgar-ECA
Germany-LBK-EN
Hungary-LBK-MN
Hungary-Koros-EN
0.29
0.30
0.31
f3 (Mbuti; France-LBKoutlier, ancient population)
SW-Iberia-EN
N-Iberia-CA
Wales-N
France-MN
Hungary-ALPc-Szatmar-MN
NE-Iberia-MLN
Scotland-N
Hungary-ALPc-Szakalhat-MN
England-N
N-Iberia-MLN
0.28
0.30
0.32
f3 (Mbuti; EF-MN1, ancient population)
Croatia-Starcevo-EN
Hungary-Koros-EN-all
NW-Iberia-MLN
Hungary-Tiszapolgar-ECA
Hungary-Sopot-LN
Germany-Baalberge-MN
Hungary-Tis-Bod-ECHA
Croatia-Sopot-MN
Germany-LBK-EN
Czech-MN
0.28
0.30
0.32
f3 (Mbuti; France-Cerny, ancient population)
SW-Iberia-EN
NE-Iberia-MLN
N-Iberia-MLN
N-Iberia-CA
Hungary-Hunyadihalom-MCHA
SE-Iberia-MLN
Germany-Baalberge-MN
Hungary-Tiszapolgar-ECA
Scotland-N
England-N
0.29
0.30
0.31
0.32
f3 (Mbuti; NF-MN2, ancient population)
Germany-Baalberge-MN
Hungary-Tis.-Bod.-ECHA
England-N
Scotland-N
Hungary-Sopot-LN
N-Iberia-CA
Iberia-Bell-Beaker-C
SW-Iberia-EN
Wales-N
N-Iberia-MLN
0.28
0.30
0.32
f3 (Mbuti; EF-MN2, ancient population)
NW-Iberia-MLN
Croatia-Starcevo-EN
NE-Iberia-CA
N-Iberia-CA
France-MN
Hungary-Sopot-LN
N-Iberia-MLN
Germany-Baalberge-MN
Hungary-ALPc-Esztar-MN
Scotland-N
0.28
0.30
0.32
f3 (Mbuti; SF-MN2, ancient population)
Germany-Baalberge-MN
N-Iberia-CA
NE-Iberia-CA
Ukraine-Trypillia-Eneolithic
Iberia-MN
Scotland-N
SW-Iberia-MLN
N-Iberia-MLN
Iberia-C
NE-Iberia-MLN
0.28
0.30
0.32



f3 (Mbuti; NF-BB, ancient population)
Croatia-Mesolithic-HG
Ukraine-N
Wales-CA-EBA
Russia-Karelia-HG
Estonia-EMN-Narva
Russia-Samara-HG
Russia-Poltavka
Netherlands-Bell-Beaker
Netherlands-BA
Romania-EN.SG
0.28
0.30
0.32
f3 (Mbuti; PEI2, ancient population)
Croatia-Mesolithic-HG
France-MN
Luxembourg-Loschbour
N-Iberia-CA
SE-Iberia-MLN
Iberia-C
Hungary-ALPc-Szakalhat-MN
SE-Iberia-CA
Hungary-MN.SG
N-Iberia-MLN
Hungary-ALPc-Tiszadob-MN
0.30
0.32
f3 (Mbuti; PEI10, ancient population)
N-Iberia-CA
Iberia-MN.SG
France-MN
N-Iberia-MLN
SW-Iberia-MLN
SE-Iberia-MLN
Hungary-Tisza-LN
NE-Iberia-MLN
Romania-EN
Iberia-C
Hungary-ALPc-Tiszadob-MN
0.30
0.32
f3 (Mbuti; EF-BA1, ancient population)
Romania-Iron-Gates-EN
Croatia-Mesolithic-HG
Germany-Blatterhohle-MN
Romania-EN.SG
N-Iberia-CA
Estonia-MN-CCC
Germany-Alberstedt-LN
Estonia-EMN-Narva
Czech-Unetice-EBA
Croatia-Vucedol
0.27
0.28
0.29
0.30
f3 (Mbuti; SF-BA1, ancient population)
NE-Iberia-CA
Luxembourg-Loschbour
Germany-Baalberge-MN
Croatia-Mesolithic-HG
SW-Iberia-EN
Croatia-Vucedol
NW-Iberia-Meso
N-Iberia-MLN
Romania-Iron-Gates-EN
Hungary-Bu-kk-MN
0.27
0.28
0.29
0.30
0.31
f3 (Mbuti; SF-BA2, ancient population)
NE-Iberia-CA
C-Iberia-CA
France-MN
N-Iberia-CA
Luxembourg-Loschbour
N-Iberia-MLN
Serbia-Iron-Gates-HG
Romania-Iron-Gates-HG
SE-Iberia-MLN
Iberia-C
0.28
0.29
0.30
0.31
f3 (Mbuti; EF-BA2, ancient population)
Romania-Iron-Gates-EN
NE-Iberia-CA
Czech-MN
C-Iberia-CA
Germany-Blatterhohle-MN
Iberia-N-EBA
Czech-Unetice-EBA
Hungary-MN.SG
Germany-Alberstedt-LN
France-MN
0.28
0.30
f3 (Mbuti; NF-IA, ancient population)
Estonia-MN-CCC
NE-Iberia-CA
Croatia-Mesolithic-HG
N-Iberia-MLN
Croatia-Vucedol
N-Iberia-CA
Switzerland-Bell-Beaker
Estonia-EMN-Narva
Wales-N
0.27
0.28
0.29
0.30
0.31

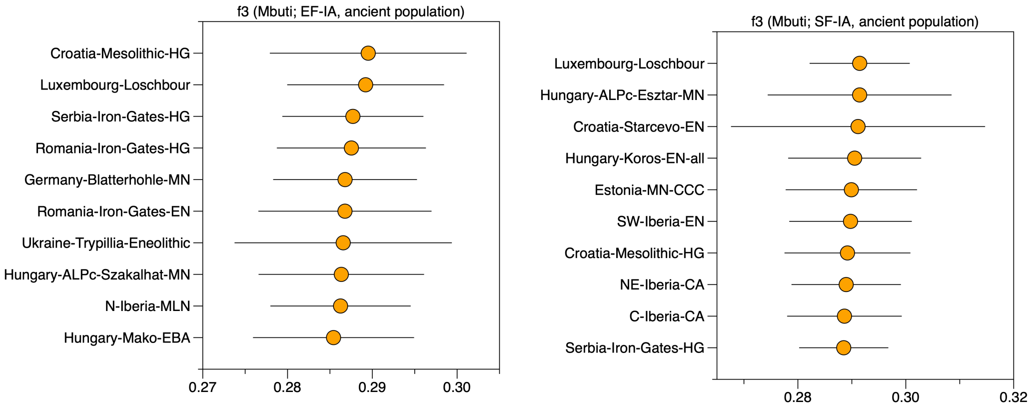


Figure S 4-3: Values of f3(Mbuti; ancient French individuals, ancient population) in ancient French populations.

4.1.4 D-statistics

D-statistics of the form D (A, B:C, D) represent a model-based approach for detecting gene flow between populations using multiple genomic loci, by testing for violation of the proposed tree topology[60]. By setting A as outgroup (see above), the D statistics tests for the genetic similarity between population B and the clade formed by C and D.

To explain patterns of genetic shift and estimate the genetic contributions of different prehistoric groups, such as the hunter-gatherers, early Anatolian farmers, and Steppe herders, we computed D-statistics of the form D(Mbuti, Test; WHG, Anatolia_Neolithic) and D(Mbuti, Test, Yamnaya_Samara, Anatolia_Neolithic). The first reflects the proportion of admixture with Western hunter-gatherers (Figure S4-4), and the latter is sensitive to the contribution of the Yamnayas to the allelic frequencies of our individuals (Figure S4-5).

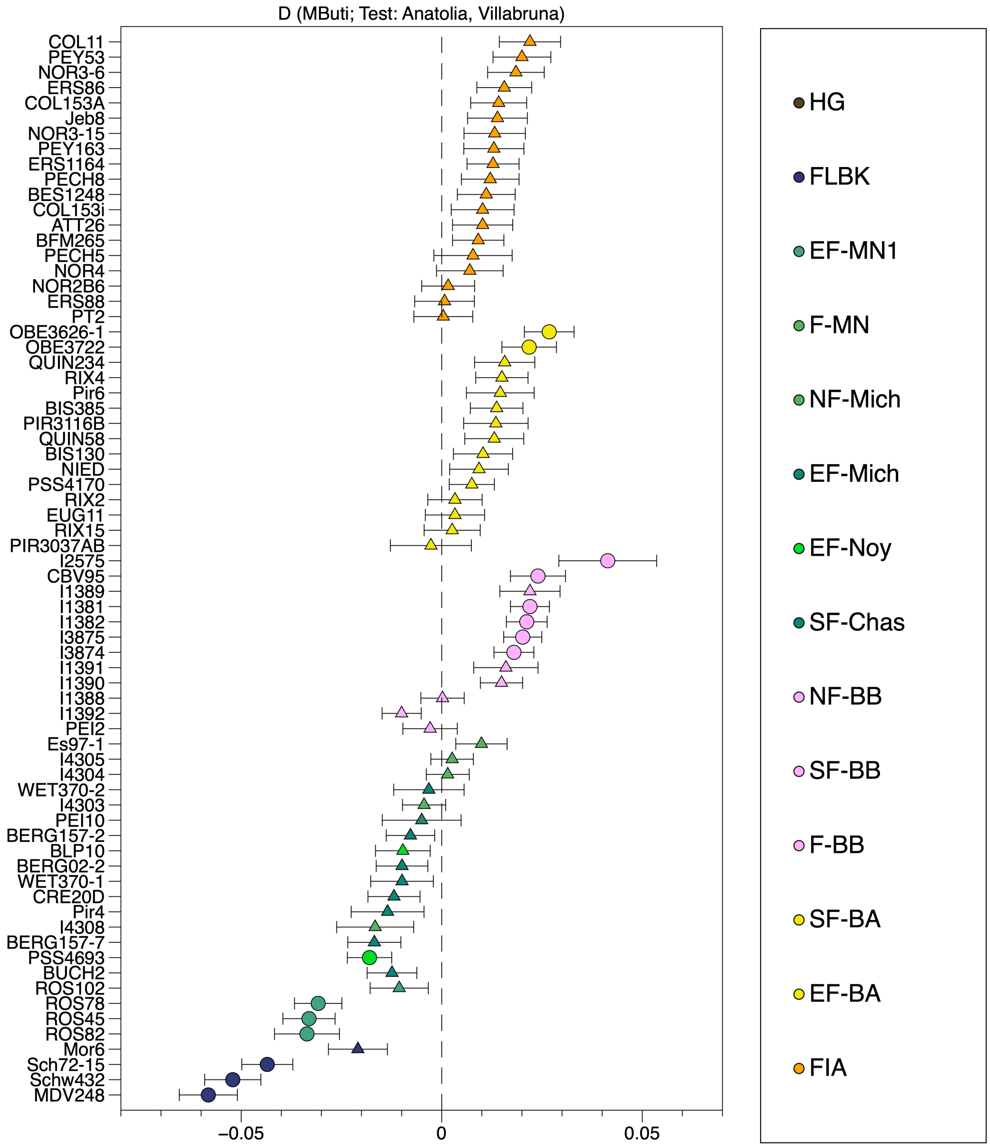


Figure S 4-4: D-statistics of the form D(Mbuti, Test: Anatolia_Neolithic, Villabruna).

Error bars represent one standard error. Symbols were used to reflect the value of the associated Z score: |Z|>3: circle; |Z|<3: triangle. Values of D(Mbuti, Test: Anatolia_Neolithic, Villabruna) for Mesolithic hunter-gatherers from Southern France ranged from 0.2183 to 0.205 (Z: 29.368 to 34.201), outside the range of any other ancient individual, and were not displayed on the figures.

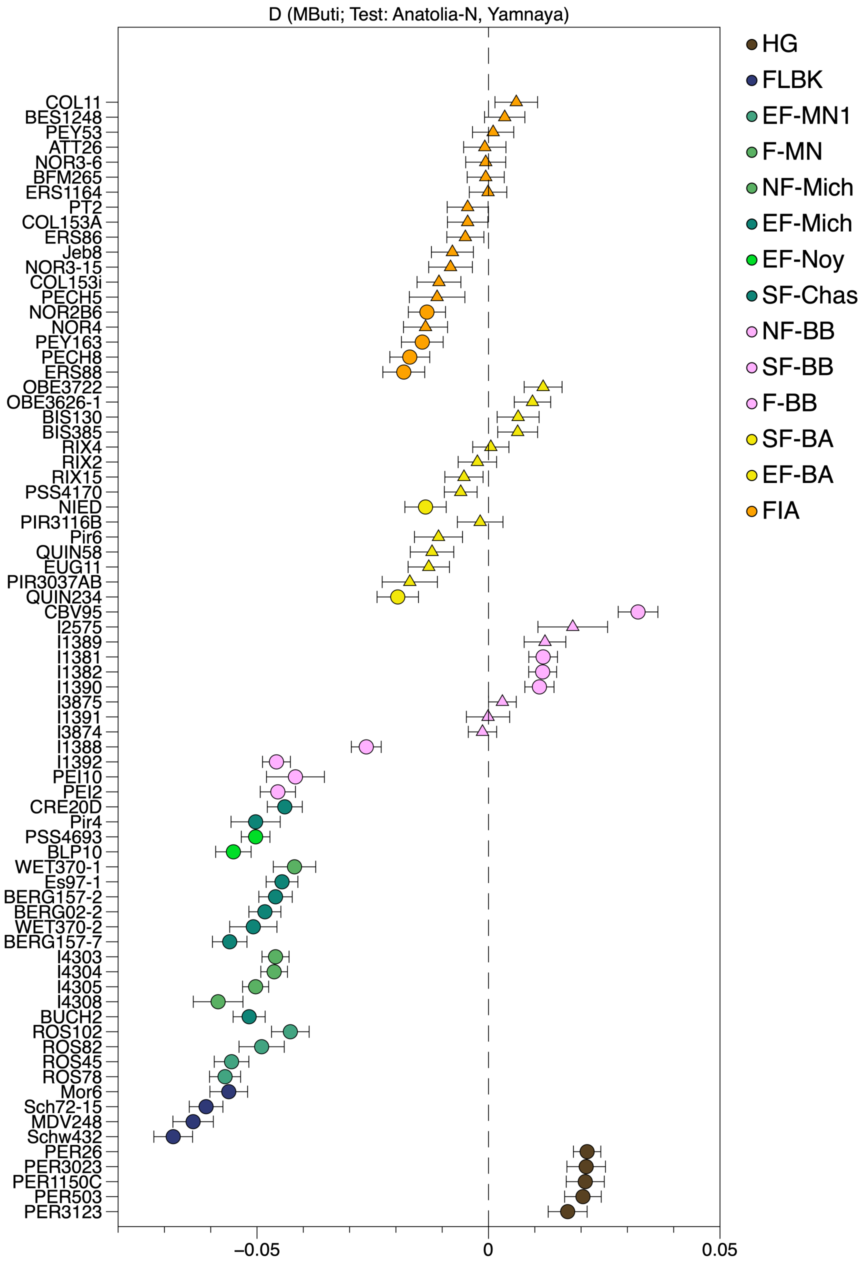


Figure S 4-5: D-statistics of the form D(Mbuti, Test: Yamnaya_Samara, Anatolia_Neolithic).

Error bars represent one standard error. Symbols were used to reflect the value of the associated Z score: |Z|>3: circle; |Z|<3: triangle.

Since the Neolithic farmers who migrated along the two different routes displayed slightly different gene pools, we investigated the origin of the Neolithic component in our individuals in order to determine from which place of Europe it originated. To assess the affinity of our Neolithic, Bronze and Iron Age groups to different Early Neolithic populations, we computed D-statistics of the form D(Mbuti, Ancient French individual; Iberia_EN, LBK_EN) where Iberia_EN and LBK_EN are Early Neolithic groups from Iberia and Germany respectively (Figure S4-6). This statistic, however, does not account for more complex scenarios where one of these populations might share alleles with another group, such as hunter-gatherers. To test this potential correlation, we used Spearman's rank correlation test, a measure of the relationship between variables when it can be described as a non-linear but monotonous function (Figure S4-7).

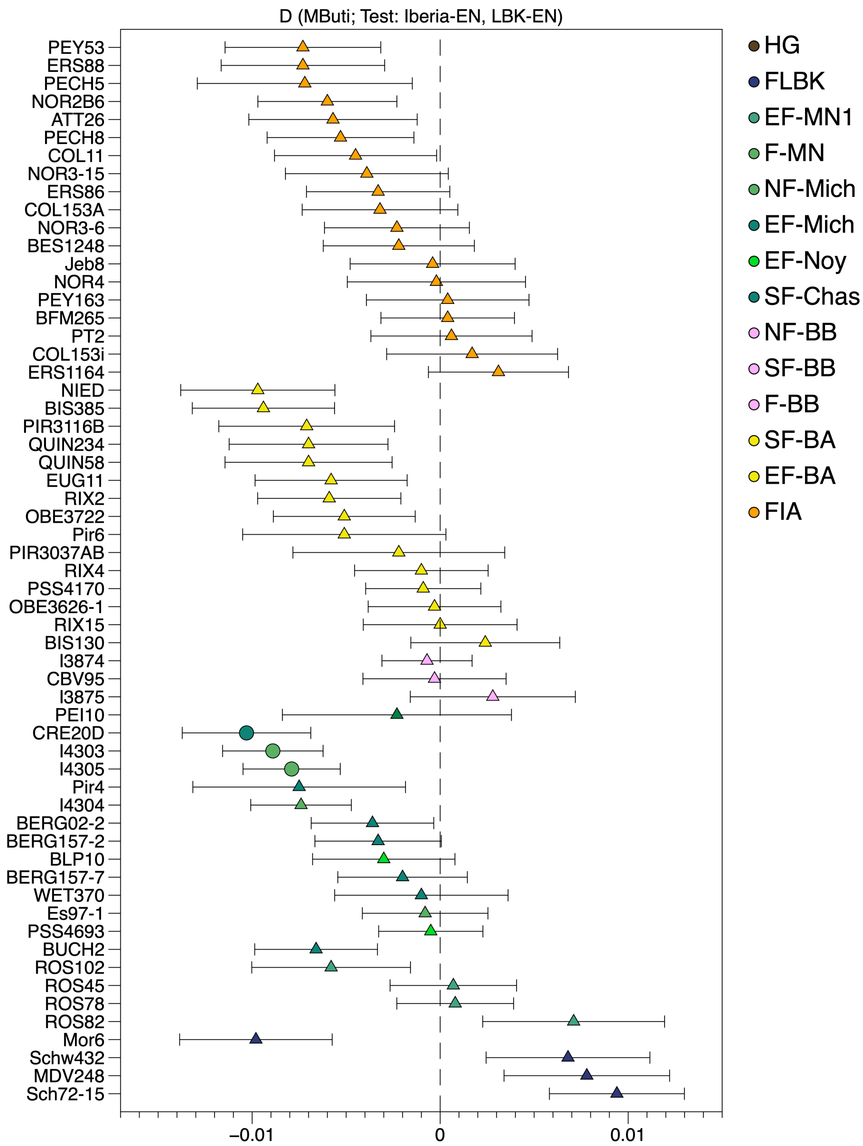


Figure S 4-6: D-statistics of the form D(Mbuti, Test: Iberia_EN, LBK_EN).

Error bars represent one standard error. Symbols were used to reflect the value of the associated Z score: |Z|>3: circle; |Z|<3: triangle.

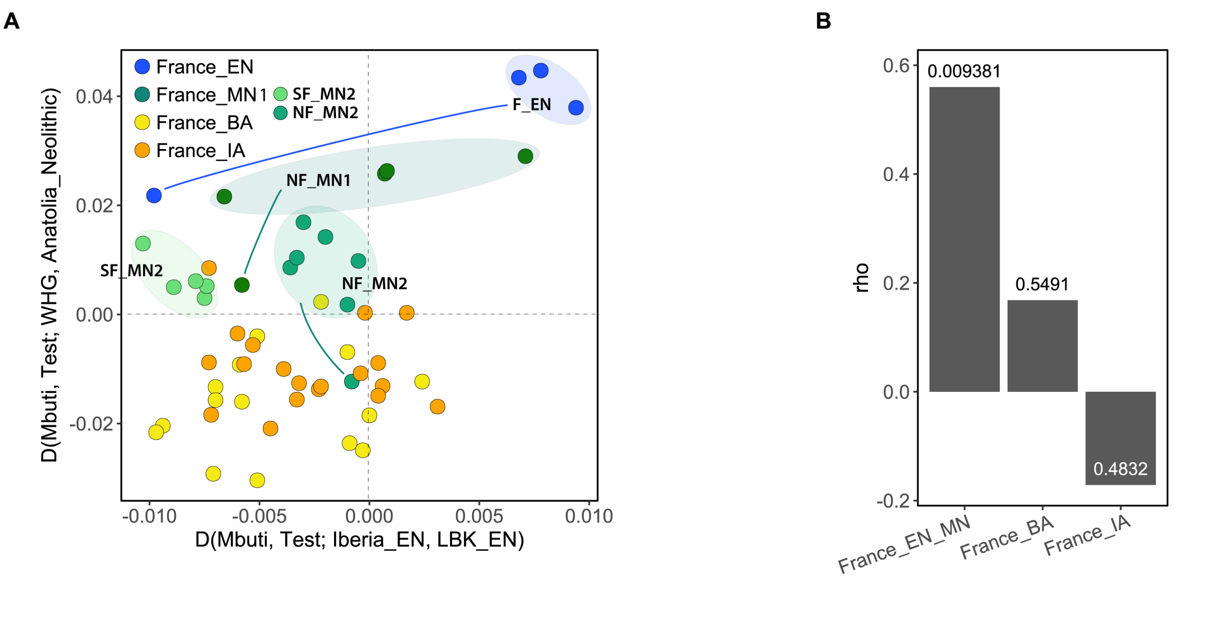
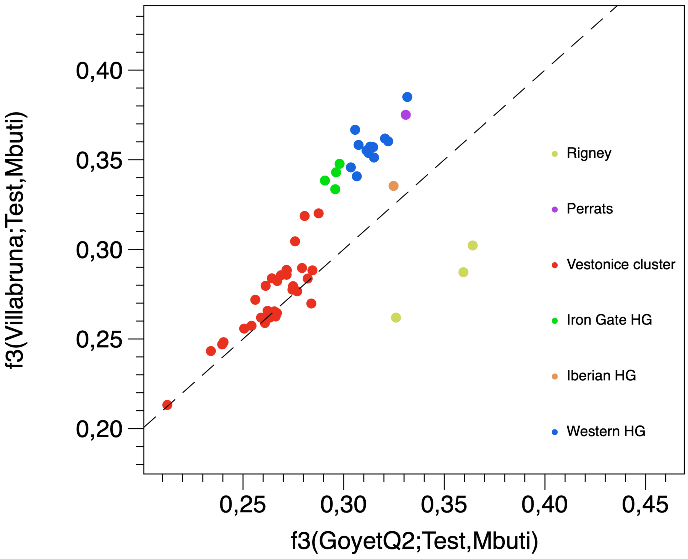


Figure S 4-7: Results from Spearman's rank correlation test.

A. Correlation was tested between the values of D-statistics of the form D(Mbuti, Test; WHG, Anatolia_Neolithic) and D(Mbuti, Test: Iberia_EN, LBK_EN) on individuals from Early and Middle Neolithic (dark blue and dark green circles), Bronze Age (yellow circles) and Iron Age individuals (orange circles). B. Histogram of the rho values for each population tested, p-values are indicated on the figure.

We investigated the affinity of our ancient samples with the different high-coverage western hunter-gatherers to determine the origin of their WHG ancestry component using f3(GoyetQ2: Test, Mbuti) (Figure S4-8).

Figure S 4-8: Biplot of f3 outgroup showing the Villabruna-like ancestry vs GoyetQ2-like ancestry in Europeans Hunter-Gatherer



SI.5 Y chromosome analysis

5.1.1 Haplogroups

Y chromosome haplogroups were determined using the Yleaf software on both shotgun sequenced and captured sample bam files [61]. Among the 73 males who provided coverage of Y chromosome SNPs, 38 allowed haplogroup assignment, 13 of which were supported by shotgun sequencing. (Dataset S10, Dataset S11).

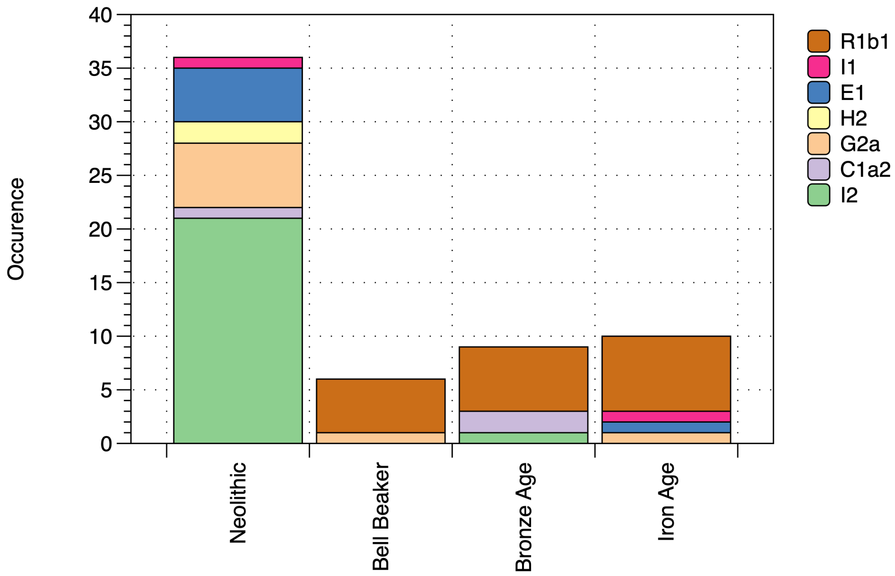


Figure S 5-1: Changes in the occurrence of 7 core Y haplogroups in the ancient French population.

5.1.2 Sex determination

Shotgun sequencing data provides an accurate identification of the biological sex of ancient remains, information that is often important to archeologists, as it is not always retrievable from fragmentary remains or juvenile individuals. Here sex assignment was performed by computing the number of alignments to the Y chromosome (nY) as a fraction of the total number of alignments to both sex chromosomes (nx + nY) according to a method published by Skoglund and colleagues[62]. The ratio of these two values is therefore RY = nY/ (nx + nY). Assuming that each sequence read from the sex chromosomes is an independent draw from two possible outcomes (Y- or X-chromosome), a 95% confidence interval (CI) was computed by a normal approximation as RY 1.96 RY (1 - RY)/ (nx + nY) (Dataset S12).

5.1.3 Kinship analysis

We looked for kinship relationships between all individuals included in this study. We used READ Relationship Estimation for Ancient DNA[63] to infer up to second degree relationships for pairs of individuals. Following the recommendation and classification, we found no relationship between our individuals except between the Mesolithic individuals. Regarding the possibility of higher homozygosity in the Mesolithic population and the smaller sample size compared to the Neolithic populations, we would suggest to take this result cautiously as a preliminary study. A deeper sequencing and increasing the sample size of Mesolithic population in order to better define allele frequency would be necessary to address properly this question of kinship. Therefore, our study suggests a first degree relationship between PER503 and PER 3123 and a second degree for both of them with PER3023.

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